

Alkaloids from *Papaver setigerum*

Chul Lee,^a Sanggil Choe,^{†,a} Jin Woo Lee, Qinghao Jin, Mi Kyeong Lee, and Bang Yeon Hwang^{*}

College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea. *E-mail: byhwang@chungbuk.ac.kr

[†]Forensic Chemistry Division, Southern District Office, National Forensic Service, Yangsan 626-815, Korea

Received January 3, 2013, Accepted January 24, 2013

Key Words : *Papaver setigerum*, Papaveraceae, Phthalideisoquinoline alkaloid

The genus *Papaver*, which belongs to the family Papaveraceae, consists of approximately 110 annual, biennial, and perennial plants distributed in central and south-western Asia, central and southern Europe, and northern Africa.¹ A variety of structurally diverse alkaloids such as benzyloisoquinoline, morphinane, aporphine, protopine, and phthalideisoquinoline has been isolated from species of Papaveraceae.²⁻⁴ Of the genus *Papaver*, only the opium poppy (*P. somniferum* L.) and *P. setigerum* D. C. are able to produce the narcotic substances such as morphine, codeine and thebaine as their secondary metabolites. Although morphine is the most powerful pain-killer, it also has very strong addictive properties. For this reason, *P. setigerum* and *P. somniferum* are controlled as illegal opium poppy in Korea. In our previous study on unknown poppy from Jeju Island, it was identified as *P. setigerum* using by metabolite profiling and genetic methods.^{5,6} *P. setigerum* has been reported to contain various benzyloisoquinoline, morphinane, and phthalideisoquinoline alkaloids.^{5,7} In this work, we have investigated the chemical constituents of the whole plants of *P. setigerum*. The present paper describes the isolation and structural characterization of a new rearranged phthalideisoquinoline alkaloid along with two known benzyloisoquinoline and a known phthalideisoquinoline alkaloids.

The EtOAc-soluble fraction of the whole plants of *P. setigerum* was successively subjected to silica gel, C18, and semipreparative HPLC on ODS yielded a new alkaloid **1** together with three known compounds, papaverine (**2**),⁸ papaveraldine (**3**),⁹ and α -noscapine (**4**).¹⁰

Compound **1**, tentatively named setigerumine I, was obtained as a light yellow amorphous powder and the UV absorption was appeared at 290 nm. The positive HRESIMS of **1** gave a $[M+H]^+$ at m/z 428.1356 (calcd 428.1345),

corresponding to the molecular formula C₂₂H₂₁NO₈. The ¹H-NMR spectrum of **1** displayed a singlet aromatic proton (δ_H 6.41, ring A), a pair of *ortho*-coupled aromatic protons (δ_H 7.48 and 7.42, ring B), a -CH-CH-CH₂- group (δ_H 4.62, 3.86, 3.05, and 2.88), a methylenedioxy group (δ_H 5.87), three methoxy groups (δ_H 4.05, 3.98, 3.66), and a *N*-methyl group (δ_H 2.86). The ¹³C- and DEPT NMR spectra of **1** yielded 22 carbon signals including those corresponding to a carbonyl carbon (δ_C 166.3), and a spiroketal carbon (δ_C 108.9). These ¹H-, ¹³C-, and DEPT NMR data indicated that **1** was closely similar with a rearranged phthalideisoquinoline alkaloid, dactylicapnosinine except for an additional methoxy group.¹¹ The skeleton of rearranged phthalideisoquinoline alkaloid, consists of unique ring C, D with C-N-O-C, moieties, and the lactone ring E, was further clarified with ¹H-¹H COSY and HMBC experiments. The five membered ring C was confirmed by -CH-CH-CH₂- correlation with ¹H-¹H COSY data. Moreover, the A-E ring system was assigned by the observed HMBC correlations from H-1 (δ_H 3.05, 2.88) to C-2, C-7, C-3a and C-7a, from H-3 (δ_H 4.62) to C-3a and C-6', and from H-1' (δ_H 7.42) to C- α . The position of methoxy groups were established by HMBC correlations from three methoxy groups (δ_H 4.05, 3.98, 3.66) to C-4', C-3', and C-4, respectively. Furthermore, methylenedioxy group (δ_H 5.87) was correlated with C-5, and C-6. The relative configuration of **1** was determined on the basis of coupling constant and NOESY experiment. The H-2 and H-3 was confirmed as *cis*- and axial orientations by a comparison of coupling constant between H-2 and H-3 ($J = 8.5$ Hz), and the observed NOE correlations.¹¹ In the NOESY spectrum, the cross peak was detected between H-3 and H-1', which is clearly indicated the relative configuration of C- α as shown in Figure 3. However, the optical rotation showed a zero value, it may be a racemate. This rearranged phthalideisoquinoline alkaloid is reported for the first time in the genus Papaveraceae.

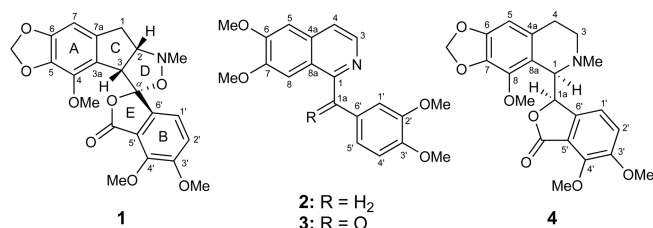


Figure 1. Structures of 1-4.

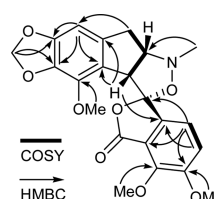


Figure 2. Key ¹H-¹H COSY, and HMBC correlations of **1**.

^aThese authors contributed equally to this work.

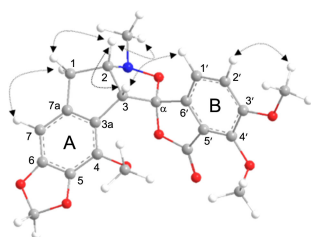


Figure 3. Key NOESY correlations of **1**.

Table 1. ^1H - and ^{13}C -NMR data of compound **1** (CD_3OD)^a

Carbon No.	1	
	δ_{H}	δ_{C}
1	3.05 (1H, dd, $J = 7.0, 17.0$ Hz) 2.88 (1H, d, $J = 17.0$ Hz)	34.7 t ^b
2	3.85 (1H, t, 8.5 Hz)	73.8 d
3	4.62 (1H, d, $J = 8.5$ Hz)	63.9 d
3a	-	121.5 s
4	-	139.4 s
5	-	133.7 s
6	-	150.9 s
7	6.41 (1H, s)	98.3 d
7a	-	135.8 s
1'	7.42 (1H, d, $J = 8.0$ Hz)	117.8 d
2'	7.48 (1H, d, $J = 8.0$ Hz)	119.2 d
3'	-	154.2 s
4'	-	147.3 s
5'	-	119.4 s
6'	-	138.3 s
C- α	-	108.9 s
-OCH ₂ O-	5.87 (2H, dd, $J = 1.0, 8.5$ Hz)	100.9 t
3'-OCH ₃	3.98 (3H, s)	55.9 q
4'-OCH ₃	4.05 (3H, s)	61.1 q
4-OCH ₃	3.66 (3H, s)	57.9 q
NMe	2.86 (3H, s)	42.6 q
-OCO-	-	166.3 s

^aAssignments aided by a combination of HMQC, HMBC, and COSY experiments. ^bCarbon multiplicity.

Experimental

General Procedures. Melting points were measured on Buchi model B-540 without correction. Optical rotations were determined with a JASCO DIP-1000 polarimeter. UV spectra were obtained on JASCO UV-550 spectrometer. NMR spectra were recorded on a Bruker DRX 500 MHz NMR spectrometer using CD_3OD and $\text{DMSO}-d_6$ as solvents with the tetramethylsilane as an internal standard. Chemical shift are presented in ppm. High resolution time-of-flight (HRTOF) and electrospray ionization (ESI) mass spectra were measured on Bruker maXis 4G and Waters QTOF micromass spectrometers, respectively. Semipreparative HPLC was performed using a Waters HPLC system equipped with two Waters 515 pumps and a 2996 photodiode array detector using a YMC J'sphere ODS-H80 column (4 μm , 150 \times 20 mm, i.d., flow rate 6 mL/min). Open column

chromatography was performed using silica gel (70-230 mesh, Merck) and Lichroprep RP-18 (40-63 μm , Merck). Thin-layer chromatography (TLC) was performed using precoated silica gel 60 F₂₅₄ (0.24 mm, Merck) plates.

Plant Materials. *P. setigerum* was collected from the Jeju Island, Korea, in May 2009. A photographic voucher specimen has been deposited at the National Forensic Service, Korea.

Extraction and Isolation. The air-dried whole plants of *P. setigerum* (0.5 kg) were pulverized at room temperature and heated at 60 °C during an hour with 0.1 N-HCl. After cooling, the filtrate was neutralized with 10% NH_4OH solution until pH 9-10, and then extracted with the EtOAc (9 L, three times) to give the EtOAc extract (13 g). The EtOAc soluble fraction was subjected to silica gel (5 \times 12 cm), eluted with CH_2Cl_2 -MeOH (100:0 to 50:50), to give 8 sub-fractions (PSE1-PSE8). Fraction PSE3 (0.9 g) was applied to a silica gel column (2 \times 10 cm), eluted with CH_2Cl_2 -MeOH (100:1, 98:1, 95:1), to yield compound **2** (13 mg). Fraction PSE6 (1.1 g) was chromatographed on silica gel with CH_2Cl_2 -MeOH (100:1, 95:1, 90:1, 80:1), to give 6 subfractions (PSE61-PSE66). Subfraction PSE62 (0.2 g) was further subjected to chromatography over RP-18 (2 \times 20 cm), eluted with H_2O -MeOH (20, 40, 60, 80%, gradient), to yield compound **3** (7 mg). Subfraction PSE64 (90 mg) was further purified by semipreparative HPLC and eluted with H_2O -ACN (33 to 62%, gradient), to yielded compound **1** (3 mg) and compound **4** (9 mg).

Setigerumine I (1): Light yellow amorphous powder; mp 184.5-186°; $[\alpha]_{\text{D}}^{25}$ 0° (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (4.31) nm; ^1H -NMR (CD_3OD , 500 MHz): see Table 1; ^{13}C -NMR (CD_3OD , 125 MHz): see Table 1; HRESI-MS m/z 428.1356 (calcd for $\text{C}_{22}\text{H}_{22}\text{NO}_8$, 428.1345).

Acknowledgments. This study was supported by funding from the National R&D program of the Ministry of Education, Science and Technology (MEST), Korea: Development of Molecular Sensing Technology (Code number 2009-0984151). The authors wish to thank the Center for Research Instruments and Experimental Facilities, Chungbuk National University for providing the NMR spectroscopic measurements.

Supporting Information. ^1H -, ^{13}C -NMR, DEPT, HSQC, HMBC, NOESY, and HRESI-MS spectra of **1** and ^1H -, ^{13}C -NMR data of compounds **2-4** are available as Supporting Information.

References

- Kadereit, J. W. *Papaveraceae*; Kubitzki, K.; Rohweeer, J. G.; Bittrich, V., Ed.; *The Families and Genera of Vascular Plants*; Springer-Verlag: Berlin, Heidelberg, Germany, 1993; Vol. 2, p 20.
- Preininger, V. *Chemotaxonomy of Papaveraceae and Fumariaceae*; Brossi, A., Ed.; The alkaloids; Academic Press: London, U. S.A., 1986; p 2.
- Preininger, V.; Novak, J.; Santavy, F. *Planta Med.* 1981, 41, 119.
- Frick, S.; Kramell, R.; Schmidt, J.; Fist, A. J.; Kutchan, T. M. *J.*

- Nat. Prod.* **2005**, 68, 666.
5. Choe, S.; Lee, E.; Jin, G.; Lee, Y. H.; Kim, S. Y.; Choi, H.; Chung, H.; Hwang, B. Y.; Kim, S. *Forensic Sci. Int.* **2012**, 222, 387.
 6. Choe, S.; Kim, S.; Lee, C.; Yang, W.; Park, Y.; Choi, H.; Chung, H.; Lee, D.; Hwang, B. Y. *Forensic Sci. Int.* **2011**, 211, 51.
 7. Slavík, J.; Slavíková, L. *Collect. Czech. Chem. Commun.* **1996**, 61, 1047.
 8. Gilmore, C. D.; Allan, K. M.; Stoltz, B. M. *J. Am. Chem. Soc.* **2008**, 130, 1558.
 9. Janssen, R. H. A. M.; Wijkens, P.; Kruk, C.; Biessels, H. W. A.; Menichini, F.; Theuns, H. G. *Phytochemistry* **1990**, 29, 3331.
 10. Marsaioli, A. J.; Magalhães, A. F.; Rúveda, E. A.; Reis, F. d. A. M. *Phytochemistry* **1980**, 19, 995.
 11. Zhang, G.; Rücker, G.; Breitmaier, E.; Nieger, M.; Mayer, R.; Steinbeck, C. *Phytochemistry* **1995**, 40, 299.
-