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NORDITERPENOID ALKALOIDS FROM THE ROOTS OF *DELPHINIUM POTANINII*

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Key Word Index—*Delphinium potaninii*; Ranunculaceae; norditerpenoid alkaloids; potanisine C; potanisine D; potanisine E.

Abstract—Three new norditerpenoid alkaloids, potanisines C, D and E, have been isolated from the roots of *Delphinium potaninii*. The structures of these compounds were derived from their spectroscopic data. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The plant *Delphinium potaninii* W. T. Wang grows in the southwestern part of China, and its roots are used in folkloric medicine for the treatment of rheumatism and neuralgia. In previous papers, we reported the structures of potanidines A and B [1], potanine [2], potanisines A and B [3], together with known alkaloids such as lycoctonine, anthranoyllycoctonine, methyllycaconitine, delsemines A and B, delavaines A and B, and takaosamine, from the roots of *D. potaninii*. Further investigation on this plant has now led to the isolation of additional new norditerpenoid alkaloids, potanisines C (**1**), D (**5**) and E (**6**). This paper deals with the isolation and characterization of these alkaloids.

RESULTS AND DISCUSSION

Potanisine C (**1**) was isolated as a homogeneous amorphous powder. Its molecular formula $C_{25}H_{39}NO_9$ was derived from its FD mass spectrum ($[M]^+$ at *m/z* 497) and ^{13}C NMR analyses. The ^1H and ^{13}C NMR spectra indicated the presence of five methoxyl groups (δ_H 3.14, 3.39, 3.42, 3.50, 3.52; δ_C see Table 1) and an *N*-formyl group (δ_H 8.84 and δ_C 178.4). Along with the ^{13}C signals for the methoxyl and *N*-formyl carbons, the ^{13}C NMR spectrum displayed 19 carbon signals. Ten of these signals (δ 70–90) were attributed to oxygenated or nitrogenated carbons. The spectral characteristics of compound **1** were indicative of norditerpenoid alkaloids, and it was considered to be a lycoctonine-type on the basis of the chemotaxonomic evidence and a comparison of the NMR properties with known compounds of this type. Thus, a proton triplet at δ 3.61 (J = 4.3 Hz) was readily assignable to the 14β-H, and it could be observed that a methoxyl group was present at C-14 (δ 80.3 *d*). The remaining four methoxyl groups were assigned to C-1 (δ 83.5 *d*), C-6 (δ 87.1 *d*), C-8 (δ 79.6 *s*), and C-16 (δ 83.7 *d*) by a comparison of the ^{13}C NMR data with those of lycoctonine (**2**) [4] and 14-*O*-methyldeltatsine (**3**) [5] (Table 1).

Attention was then focused on the assignment of the remaining oxygenated and nitrogenated carbons. Among the ^{13}C NMR signals appearing between δ 70 and 90, two (δ 71.1 *d* and 57.6 *t*) were attributed to C-17 and C-19, respectively. A singlet at δ 90.5 was comparable with that of C-7 in norditerpenoid alkaloids bearing 6,8-di-OCH_3 and 7-OH groups (such as delbotine and delboxine [6]), and a methylene carbon (δ 78.1 *t*) was assigned to C-18 which bears a methoxyl group. Lastly, a carbon signal overlapped with the C-17 signal at δ 71.1. For this CH_2 signal, there were several possible assignments, such as C-3, C-12 or C-15. By comparison with known structures such as **2** [4] and **3** [5], the oxygenated carbon was assigned to C-15 for the following reasons. In the ^1H NMR spectrum of potanisine C, a two-proton multiplet at δ 1.5–1.7 was readily assigned to 3-CH_2 [7], indicating that C-3 was unsubstituted. The other possibility, C-12, was ruled out because C-10 resonated at δ 42.3 which would have otherwise shown a downfield shift of around 5-6 ppm (β-effect). Therefore a hydroxyl group was assigned to C-15. Acetylation of potanisine C with acetyl anhydride and pyridine at room temperature produced a diacetyl derivative (**4**) [δ 2.03 and 2.14 (each 3H, *s*, OAc)], confirming the presence of two primary and secondary hydroxyl groups.

The ^{13}C NMR data for potanisine C (**1**) were similar to those for lycoctonine (**2**) and 14-*O*-methyldeltatsine (**3**) (Table 1). It was noted that the chemical shift of C-2 in **1** was shifted upfield, due to a shielding effect of

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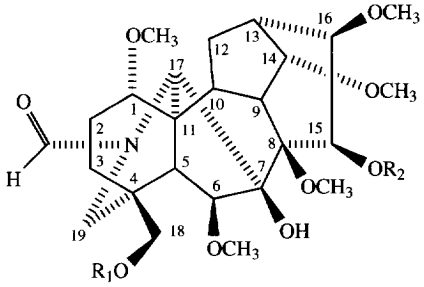
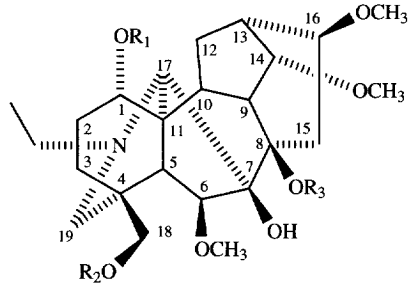
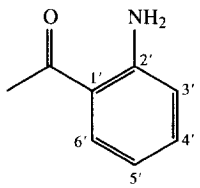
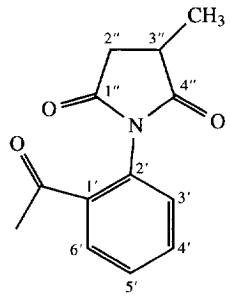


1 $R_1 = R_2 = H$
4 $R_1 = R_2 = Ac$



2 $R_1 = CH_3$; $R_2 = R_3 = H$
3 $R_1 = H$; $R_2 = R_3 = CH$

5 $R_1 =$  $R_2 = H$

6 $R_1 =$  $R_2 = H$

the *N*-formyl group. Finally the configuration of the 15-OH group was examined. Since it is known that the conformation of the D-ring in lycoctonine-type alkaloids is the boat form, the values of $J_{15,16}$ were similar regardless of the C-15 configuration. For example, the $J_{15,16}$ values for 15α-hydroxyneoline (senbusine C) and 15β-hydroxyneoline were reported to be 6 Hz and 8 Hz, respectively [8, 9]. We then compared the carbon shifts for C-15 and C-16 among related structures. It was obvious that they consistently resonated at *ca.* δ 77 and 91, respectively, in compounds bearing 15α-OH and 16β-OCH_3 groups (the C-16 signal can be deshielded to *ca.* δ 95 when 13-OH is present) [8, 10–12]; whereas the chemical shifts for C-15 and C-16 in 15β-hydroxylneoline were found at δ 68 and 84, respectively [9]. After considering the shift values of C-15 and C-16 of potanisine C (δ 71 and 84), the configuration of the 15-OH was deduced to be β as shown in **1**.

Potanisine D (**5**) was assigned to the molecular formula $C_{32}H_{44}N_2O_{10}$ by HRFAB-mass spectrometry (*m/z* 599.3371 $[M-OH]^+$) and ^{13}C NMR studies. Its ^1H and ^{13}C NMR spectra revealed the presence of an anthranoyl group (δ_H 6.65, 6.65, 7.29, 7.83; δ_C 109.9 *s*, 116.3 *d*, 116.8 *d*, 131.1 *d*, 134.4 *d*, 150.8 *s*, 167.0 *s*) [13, 14], along with five methoxyl groups (δ_H 3.16, 3.41, 3.44, 3.52, 3.56; δ_C 53.0, 56.1, 56.5, 57.7, 60.6), and an *N*-formyl group [δ_H 8.70 and δ_C 175.8]. By a comparison with the spectral data of potanisine C (**1**), the structure of potancine D was determined to be an anthranoyl derivative of **1** at C-18. In accordance with the presence of an ester group, the chemical shift value of C-18 was shifted upfield to δ 64.4 in the spectrum of potanisine D. When the compound was hydrolysed in 5% methanolic NaOH soln, potanisine C (**1**) was identified as the major product by TLC and EI-mass spectrometry analyses. These results are in accordance with the designation of structure **5** for potanisine D.

The structure of potanisine E (**6**), the third new alkaloid, was derived by similar approaches. Potanisine E was isolated as an amorphous substance, and its molecular formula $C_{37}H_{48}N_2O_{12}$ was established by EI-mass spectrometry (M^+ *m/z* 712) in combination with the ^{13}C NMR data. Analogous to the spectra of compounds **1** and **5**, the ^1H and ^{13}C NMR spectra of potanisine E revealed the presence of five methoxyl groups (δ_H 3.13, 3.41, 3.44, 3.55, 3.57; δ_C 53.0, 56.1, 56.5, 57.7, 60.6), and an *N*-formyl group (δ_H 8.62 and δ_C 175.1). In addition, signals attributable to an ester of 2-(methyl-succinimido)-benzoic acid (δ_H 7.29, 7.57, 7.71, 8.13; δ_C see Table 1) [15–17] were observed. The ^{13}C NMR data clearly indicated that the alkamine part of potanisine E was identical to potanisine C. Thus, potanisine E possessed an ester group at C-18 as

Table 1. ^{13}C NMR data of potanisines C-E (**1**, **5**, **6**), lycoctonine (**2**), and 14-*O*-methyldeltatsine (**3**)

C	**1**	**2** [4]	**3** [5]	**5**	**6**
1	83.5 *d**	84.2	72.3	83.6 *d**	83.6 *d**
2	18.7 *t*	26.1	27.0	18.7 *t*	18.7 *t*
3	31.0 *t*	31.6	29.7	31.2 *t*	31.4 *t*
4	48.2 *s*	38.6	37.2	46.8 *s*	46.5 *s*
5	40.6 *d*	43.3	39.0	40.9 *d*	41.0 *d*
6	87.1 *d*	90.6	91.2	86.2 *d*	85.7 *d*
7	90.5 *s*	88.3	91.2	91.5 *s*	91.6 *s*
8	79.6 *s*	77.5	82.1	79.6 *s*	79.5 *s*
9	47.6 *d*	49.7	49.1	46.6 *d*	46.7 *d*
10	42.3 *d*	46.1	44.9	42.3 *d*	42.2 *d*
11	50.6 *s*	48.9	49.1	50.3 *s*	50.5 *s*
12	28.4 *t*	28.8	29.4	28.4 *t*	28.4 *t*
13	37.8 *d*	38.0	36.7	38.3 *d*	38.0 *d*
14	80.3 *d*	84.0	84.1	80.5 *d*	80.1 *d*
15	71.1 *d*	33.7	30.2	71.3 *d*	71.5 *d*
16	83.7 *d**	82.7	83.2	83.9 *d**	84.0 *d**
17	71.1 *d*	64.8	66.1	71.3 *d*	71.5 *d*
18	78.1 *t*	67.6	78.1	64.4 *t*	65.4 *t*
19	57.6 *t*	52.9	57.6	58.5 *t*	58.5 *t*
$N\underline{C}H_2CH_3$	—	51.1	50.4	—	—
$NCH_2\underline{C}H_3$	—	14.1	13.7	—	—
NCHO	178.4 *s*	—	—	175.8 *s*	175.1 *s*
1-OCH_3	55.9 *q*	55.7	—	56.1 *q*	56.1 *q*
6-OCH_3	60.1 *q*	57.5	59.5	60.6 *q*	60.6 *q*
8-OCH_3	52.6 *q*	—	50.8	53.0 *q*	53.0 *q*
14-OCH_3	57.6 *q*	58.0	57.6	57.7 *q*	57.7 *q*
16-OCH_3	56.5 *q*	56.2	56.4	56.5 *q*	56.5 *q*
18-OCH_3	—	—	59.5	—	—
18-OC=O	—	—	—	167.0 *s*	163.7 *s*
1′	—	—	—	109.9 *s*	127.5 *s*
2′	—	—	—	150.8 *s*	132.4 *s*
3′	—	—	—	116.8 *d*	129.4 *d*
4′	—	—	—	134.4 *d*	133.5 *d*
5′	—	—	—	116.3 *d*	131.4 *d*
6′	—	—	—	131.1 *d*	129.5 *d*
1″	—	—	—	—	180.2 *s*
2″	—	—	—	—	37.0 *t*
3″	—	—	—	—	35.3 *d*
4″	—	—	—	—	176.0 *s*
5″	—	—	—	—	16.4 *q*

*Assignments may be interchanged.

depicted in **6**. It is interesting to note that potanisines C, D and E are the first examples of lycoctonine-type norditerpenoid alkaloids containing both an *N*-formyl and a 15-hydroxyl group.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer in $CDCl_3$ using TMS as int. standard. FD-MS and HRFAB-MS were recorded on a Hewlett Packard 5890 Model II or a Jeol JMS-11 × 110 spectrometer. Adsorption chromatography was performed with silica gel H, TLC with silica gel G.

Plant material. Roots of *Delphinium potaninii* were collected in Peng County, Sichuan Province, China in September 1991, and taxonomically identified by Prof. W. T. Wang (Institute of Botany, Chinese Academy of Sciences, Beijing). Voucher specimens of the plant have been deposited in the herbarium of the School of Pharmacy, West China University of Medical Sciences.

Extraction and separation of alkaloids. Powdered roots (15 kg) of *D. potaninii* were percolated with 0.015% HCl (110 l). The soln was treated with resin (dry wt 1.25 kg), and the resin was washed repeatedly in a suction filter with deionized water and then air-dried. The resin was then thoroughly mixed with 10% ammonium water (total amount 4.3 l) and extracted in a specially designed extractor with Et_2O reflux for 1 week. Crude total alkaloids (133.4 g) were obtained from the ethereal extract.

Using a pH gradient method, a portion of total alkaloids (20 g) was separated into three parts, part A (pH 5, 16.8 g), part B (pH 7, 1.2 g) and part C (pH 8–9, 2.0 g). Part A was chromatographed on silica gel

and eluted with $CHCl_3$–MeOH (95:5). Column frs (50 ml each) were monitored by TLC detected with Dragendoff's reagent. Frs showing similar profiles were combined to give fr. 1 (6.6 g), fr. 2 (4.64 g) and fr. 3 (500 mg). Fr. 1 was redissolved in Et_2O and the insoluble residue (2 g) was chromatographed using $CHCl_3$–MeOH (8:2) to afford potanisine E (**6**, 20 mg, 0.0009% yield).

Another batch of the total alkaloids (40 g) was successively acidified to pH 2.5 and pH 1, each extracted with $CHCl_3$ to give frs C (34 g) and D (5.2 g), respectively. Fr. C was chromatographed using $CHCl_3$–MeOH (9:11) to afford four combined frs. The last fr. (439 mg) was further sepd on silica gel eluted with $CHCl_3$–MeOH (95:5), to yield the purified alkaloid potanisine C (**1**, 20 mg, 0.0005% yield). Fr. D was chromatographed repeatedly using $CHCl_3$–MeOH mixtures to give potanisine D (**5**, 10 mg, 0.00025% yield).

Potanisine C (**1**). Amorphous powder; $[\alpha]_D$ +45.5° ($CHCl_3$; *c* 1.10); FD-MS *m/z*: 497 $[M]^+$, 481, 450. ^1H NMR (200 MHz, $CDCl_3$): δ 3.14, 3.39, 3.42, 3.50, 3.52 (each 3H, *s*, 5 × OCH_3), 3.61 (1H, *t*, J = 4.3 Hz, 14β-H), 8.84 (1H, *s*, NCHO); ^{13}C NMR (50 MHz, $CDCl_3$), see Table 1.

Acetylation of potanisine C. To potanisine C (5 mg) was added Ac_2O (0.5 ml) in pyridine (1 ml). The resulting soln was allowed to stand at room temp overnight. After removal of excessive solvent under red. pres., a residue was obtained. Deionized H_2O (10 ml) was added to the residue and alkalinized with ammonium water to pH 9, followed by extraction with $CHCl_3$ (10 ml × 3). The combined organic layers were dried over Na_2SO_4, filtered, and evapd to give a powder substance (**4**) showing a single spot on TLC. ^1H NMR (200 MHz, $CDCl_3$): δ 2.03, 2.14 (each 3H, *s*, 2 × OAc), 3.15, 3.40, 3.43, 3.50, 3.57 (each 3H, *s*, 5 × OCH_3), 3.62 (1H, *t*, J = 4.3 Hz, 14β-H), 8.64 (1H, *s*, NCHO).

Pontanisine D (**5**). Amorphous powder; $[\alpha]_D$ +32.1° ($CHCl_3$; *c* 0.70); EI-MS *m/z*: 599 $[M-OH]^+$, 569 $[M-OH-OCH_3]^+$; HRFAB-MS *m/z*: 599.3371 $[M-OH]^+$ (requires 599.2968 for $C_{32}H_{43}N_2O_9$). ^1H NMR (200 MHz, $CDCl_3$): δ 3.16, 3.41, 3.44, 3.52, 3.56 (each 3H, *s*, 5 × OCH_3), 3.64 (1H, *t*, J = 4.4 Hz, 14β-H), 6.65, 6.65, 7.29, 7.83 (each 1H, *m*, aromatic protons), 8.70 (1H, *s*, NCHO). ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

Potanisine E (**6**). Amorphous powder; $[\alpha]_D$ +13.0° ($CHCl_3$; *c* 0.10); EI-MS *m/z*: 712 $[M]^+$, 681 $[M-CHO]^+$, 665 $[M-CHO-H_2O]^+$, 652 $[M-CHO-OCH_3]^+$ 651 $[M-CHO-CH_3OH]^+$. ^1H NMR (200 MHz, $CDCl_3$): δ 3.13, 3.41, 3.44, 3.55, 3.57 (each 3H, *s*, 5 × OCH_3), 3.61 (1H, *t*, J = 4.3 Hz, 14β-H), 7.29, 7.57, 7.71, 8.13 (each 1H, *m*, aromatic protons), 8.62 (1H, *s*, NCHO). ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

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REFERENCES

1. Pu, H. Y. and Wang, F. P. (1994) *Acta Pharm. Sinica* **29**, 689.
2. Pu, H. Y. and Wang, F. P. (1994) *Chinese Chem. Letters* **5**, 939.
3. Pu, H. Y., Xu, Q. Y., Wang, F. P. and Che, C. T. *Planta Med.*, in press.
4. Pelletier, S. W., Mody, N. V., Sawhney, R. S. and Bhattacharyya, J. (1977) *Heterocycles* **7**, 327.
5. Joshi, B. S., Glinski, J. A., Chokshi, H. P., Chen, S. Y., Srivastava, S. K. and Pelletier, S. W. (1984) *Heterocycles* **22**, 2037.
6. Jiang, Q. P. and Sung, W. L. (1985) *Heterocycles* **23**, 11.
7. Wang, F. P. and Fang, Q. C. (1985) *Acta Pharm. Sinica* **18**, 514.
8. Konno, C., Shirasaka, M. and Hikono, H. (1982) *J. Nat. Prod.* **45**, 128.
9. Wang, F. P. and Fang, Q. C. (1983) *Plant Med.* **47**, 39.
10. Wang, H. C., Lao, A., Fujimoto, Y. and Tatsuno, T. (1985) *Heterocycles* **23**, 803.
11. Bando, H., Wada, K., Watanabe, M., Mori, T. and Amiya, T. (1985) *Chem. Pharm. Bull.* **33**, 4717.
12. Hikino, H., Kuroiwa, Y. and Konno, C. (1983) *J. Nat. Prod.* **46**, 178.
13. Desai, H. K., Joshi, B. S. and Pelletier, S. W. (1985) *Heterocycles* **23**, 2483.
14. Pelletier, S. W., Kulanthaivel, P. and Olsen, J. D. (1989) *Heterocycles* **28**, 107.
15. Kulanthaivel, P. and Benn, M. (1985) *Heterocycles* **23**, 2515.
16. Pelletier, S. W., Desai, H. K., Kulanthaivel, P. and Joshi, B. S. (1987) *Heterocycles* **26**, 2835.
17. Pelletier, S. W., Kulanthaivel, P. and Olsen, J. D. (1989) *Phytochemistry* **28**, 1521.