Tetrahedron Vol. 45, No. 23, pp. 7281 to 7286, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

NEW STEROIDAL ALKALOIDS FROM FRITILLARIA USSURIENSIS MAXIM. PINGBEINONE AND HEILONINE

Yukie Kitamura, Makoto Nishizawa, and Koh Kaneko*
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan
Motoo Shiro
Shionogi Research Laboratory, Shionogi & Co., Fukushima Ku, Osaka, Japan
Yuh-Pan Chen and Hong-Yen Hsu
Oriental Healing Arts Institute, 1945 Palo Verde Avenue, suite 208, Long Beach, California 90815, U.S.A.

(*Received in Japan* 29 *June* 1989)

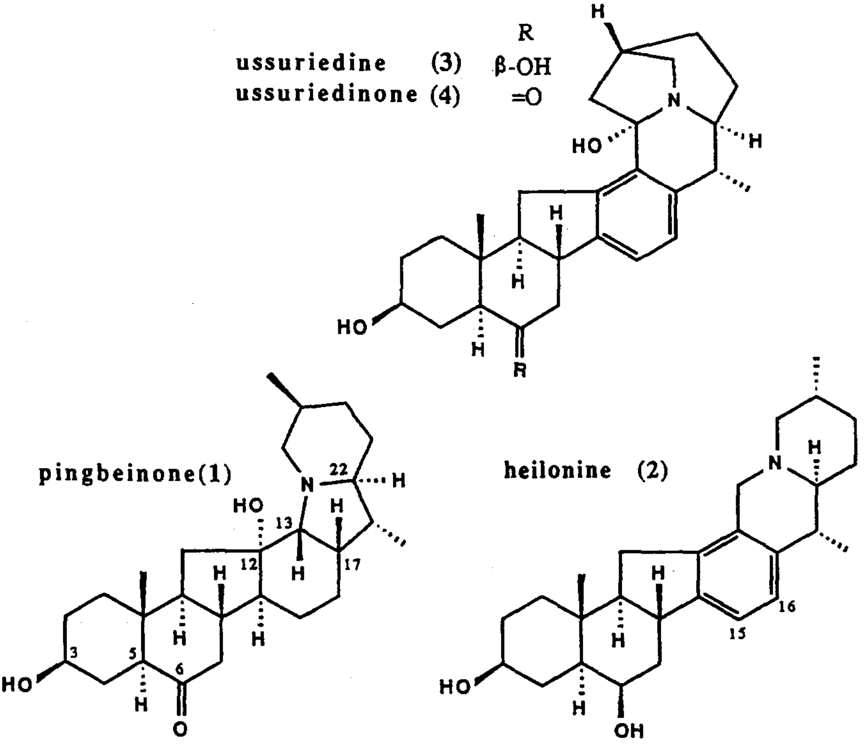
Two novel alkaloids, pingbeinone (**1**) and heilonine (**2**) were isolated from *Fritillaria ussuriensis* and their structures were determined on the basis of spectral data and X-ray crystallographic analysis. Pingbeinone is a novel C_{26} steroidal alkaloid having a skeleton lacking C-18 of C-nor D-homo steroidal alkaloid.

"Bei-mu" is an important Chinese herbal drug which has been used as an antitussive, sedative and expectorant.1) In the course of our study on the alkaloids in "Bei-mu", we have been studying *Fritillaria ussuriensis* Maxim. (Ping-bei-mu), which is cultivated in Hei-Long-Jiang province in China. In the previous paper2), we have reported the two new steroidal alkaloids having a seven ring skeleton, ussuriedine (**3**) and ussuriedinone (**4**) (Scheme). In this paper, we describe the isolation and structural elucidation of additional two new steroidal alkaloids from *F. ussuriensis*.

Results and Discussion

Pingbeinone (**1**)3) was obtained as white needles from MeOH. The mass (MS) spectrum of **1** revealed the molecular ion peak (M^+) at *m/z* 415 and the base peak at *m/z* 164. The molecular formula of **1** ($C_{26}H_{41}NO_3$), obtained by high resolution mass (HR-MS) spectrum of the M^+ ion peak, was one carbon less than those of known C-nor D-homo steroidal alkaloids. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** also exhibited 26 carbon signals (three CH_3, ten CH_2, ten CH, and three quaternary carbons). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed one singlet methyl signal (δ0.70, Me_{19}) and two secondary methyl signals at (δ0.86,*J*=7.0Hz,

Me_{21} and δ0.92, J=6.8Hz, Me_{27}). The high field shifted Me_{19} implied the presence of C-6 keto function. One hydroxymethine proton at δ 3.60 ($W_{1/2}$=23Hz) was assigned to 3α-H, based on the chemical shift and the half height width. The analysis of ^{1}H-^{1}H, ^{1}H-^{13}C correlation spectrometry (COSY) and ^{1}H-^{13}C long-range COSY spectra revealed the bold lines parts in Fig. 1 as partial structures. The C-13 (δ_C 73.1, δ_H 2.89), C-22 (δ_C 63.5, δ_H 2.67) and C-26 (δ_C 58.4, δ_H 2.79, 2.40) were shown to be connected with a nitrogen atom by the LSPD (long-range selective proton decoupling) experiment. Irradiation at δ_H 2.89 (H-13) affected the signals of C-20 and C-22. Irradiation at δ_H 2.79 (H-26) affected the signals of C-13, C-22, C-25 and C-24. And irradiation at δ_H 2.67 (H-22) affected the signals of C-13, C-17, C-20 and C-26. The LSPD experiment also exhibited the connectivity between C-24 and C-25, and the structure of 1 was assumed to be as shown in Scheme. Finally, the absolute structure of 1 was confirmed by X-ray crystallographic



Scheme

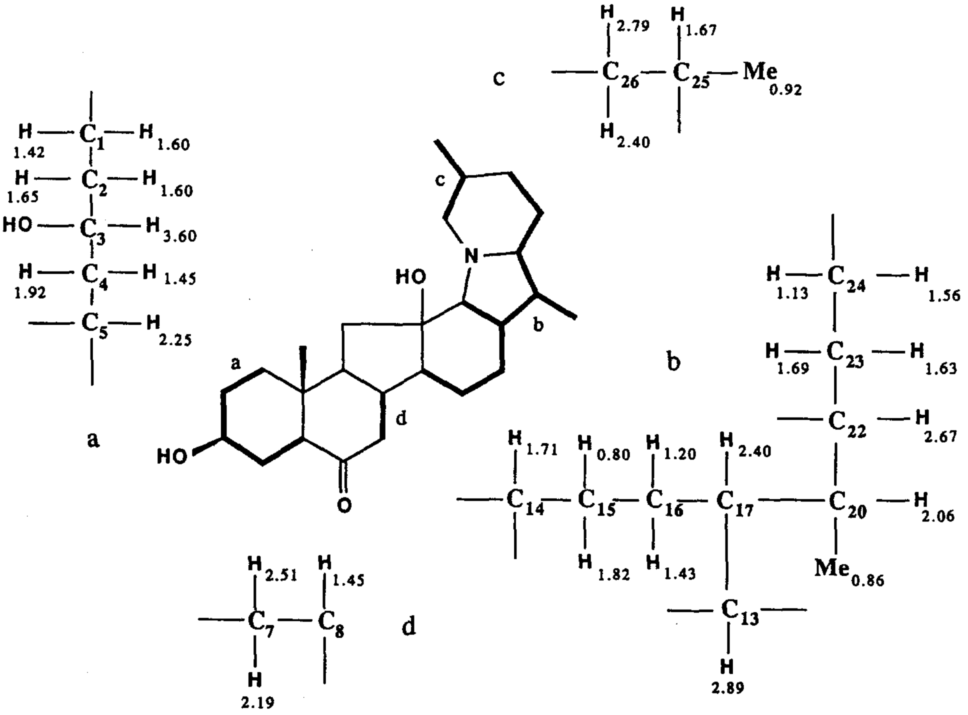


Fig. 1 Partial Strucutres of 1
Bold lines indicate connectiviteis
from ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments.

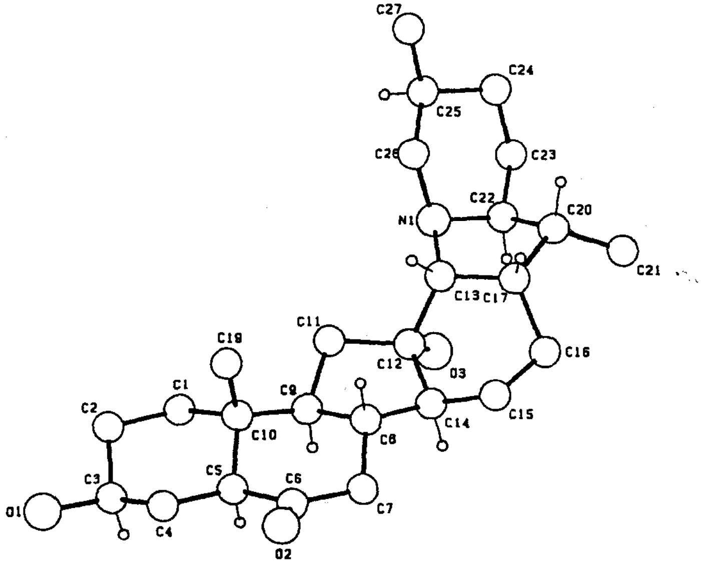


Fig. 2 Perspective Drawing of 1

Table ^1H-,^{13}C-NMR chemical shifts of 2a ($CDCl_3$)

Carbon No.	δ_C	δ_H
1	38.4	1.23, 1.65
2	27.1	1.65, 1.87
3	73.4	4.78 (septet, J=5Hz)
4	30.4	1.65, 1.76
5	47.1	1.41
6	74.0	5.10 (m, $W_{1/2}$=8Hz)
7	34.2	1.57 (dt, J= 3.0, 13.8Hz), 2.47 (dd, J=3.5, 14.0Hz)
8	39.0	3.12 (dt, J= 7,12.2Hz)
9	60.5	1.62
10	35.7	——
11	28.6	2.50 (br.t, J=13.6Hz), 2.57 (dd, J=7.1, 14.5Hz)
12	142.8	——
13	136.7	——
14	139.0	——
15	120.2	6.96 (d, J=7.8Hz)
16	125.2	7.08 (d, J=7.8Hz)
17	129.5	——
18	55.8	3.32 (d, J=15.0Hz), 3.92 (d, J=15.0Hz)
19	14.9	1.16 (s)
20	38.7	2.84 (quintet, J=7Hz)
21	19.49	1.29 (d, J=6.9Hz)
22	65.5	1.99
23	31.5	1.47, 2.15 (br.dd, J= 2.7, 13.7Hz)
24	32.9	1.04 (qd, J= 4, 11.8Hz), 1.85
25	30.5	1.88
26	64.0	1.89, 3.20 (br.d, J=10Hz)
27	19.57	0.93 (d, J=5.4Hz)
acetate	170.5x2	——
	21.27	2.04
	21.34	2.06

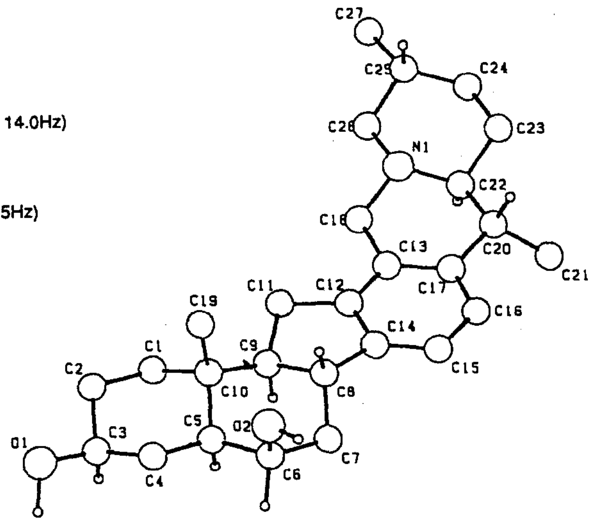


Fig. 3 Perspective Drawing of 2

analysis of its hydrogeniodide (Fig. 2)[4].

Compound **2** was obtained as colorless prisms from MeOH, and was named heilonine from the name of province, Hei-Long-Jiang, where *F. ussuriensis* was harvested. The MS spectrum of **2** revealed the ion peaks at *m/z* 409 (M^+), 394 (base peak), 312, 294 and 170. The HR-MS spectrum indicated the molecular formula of **2** as $C_{27}H_{39}NO_2$. The index of hydrogen deficiency of **2** is 9, which is one unit less than that of ussuriedine (**3**). As **2** was hard to dissolve in most of organic solvents, the spectral data of its diacetate (**2a**) was used for the structural elucidation. The ^1H-NMR spectrum of **2a** revealed one tertiary methyl (δ 1.16, Me_{19}), two secondary methyl (δ 0.93, J=5.4Hz, Me_{27} and δ 1.29, J=6.9Hz, Me_{21}) and two hydroxymethine protons (δ 4.78, septet, J=5Hz, 3α-H and δ 5.10, m, $W_{1/2}$=8Hz, 6α-H). Notably, two protons at δ 6.96 and 7.08, coupled each other by J=7.8Hz, were assignable to the ortho-protons of aromatized D ring; two methine and four quaternary carbons were observed in the aromatic region. Analysis of ^1H-^1H COSY spectrum suggested the structure of **2** as shown in Scheme (Table 5). The proposed structure of **2** was determined by X-ray crystallographic analysis as shown in Fig. 3, presuming the Me_{19} is β-axial. [5)]

Heilonine (**2**) has the ussuriedine (**3**) related structure without C-C bond between C-18 and C-27, however, the lone pair of nitrogen has the opposite orientation to that of H-22. Compound **2** is not likely to be an intermediate product in biosynthetic pathway of **3**, but must be a key compound in it. Pingbeinone (**1**) has a novel C_{26} steroidal alkaloid lacking C-18 of 5α-cevane skeleton, and is the second example of C_{26} steroidal alkaloid from *Fritillaria* genus. Heilonine is the third 5α-cevane alkaloid having aromatized D-ring. As *F. ussuriensis* was shown to contain characteristic alkaloids, especially **1** and **3**, it is interesting to clarify the biogenesis of *F. ussuriensis* .

Experimental.

General method was shown in previous paper. [2)]

Isolation; Details are in reference 2). Pingbeinone (**1**, 60.2mg) and heilonine (**2**, 28.6mg) were obtained from the free alkaloids fraction.

Pingbeinone (1); mp. 200-202°C., HR-MS; $C_{26}H_{41}NO_3$ found: 415.3076, calcd.: 415.3086., EI-MS (*m/z*); 415 (M^+), 400, 220, 164 (base peak), 112., $[\alpha]_D$ -22° (*c* 0.80, $CHCl_3$)., IR $\upsilon_{max}(CHCl_3)$ cm^{-1}; 3500(OH), 2800 (*trans*-indolidine), 1730 (carbonyl)., ^1H-NMR (400MHz, $CDCl_3$) δ; 0.70 (3H, s, 19-H), 0.86 (3H, d, *J* =7.0Hz, 21-H), 0.92 (3H, d, *J* =6.8Hz, 27-H), 3.60 (1H, m, $W_{1/2}$=23Hz, 3α-H)., ^{13}C-NMR (100MHz, $CDCl_3$) δ; 37.2 (C-1), 30.4 (C-2), 70.3 (C-3), 30.0 (C-4), 56.4 (C-5), 211.2 (C-6), 46.4 (C-7), 47.9 (C-8), 52.5 (C-9), 38.3 (C-10), 44.5 (C-11), 77.5 (C-12), 73.1 (C-13), 52.3 (C-14), 26.9 (C-15), 21.4 (C-16), 42.0 (C-17), 12.6 (C-19), 36.1 (C-20), 13.5 (C-21), 63.5 (C-22), 23.8 (C-23), 28.4 (C-24), 30.6 (C-25), 58.4 (C-26), 20.0 (C-27).

Heilonine (2); mp. 284-286°C., HR-MS; $C_{27}H_{39}NO_2$ found: 409.2984, calcd.: 409.2980., EI-MS (*m/z*); 409 (M^+), 394 (base peak), 312, 294, 170., UV λ_{max}(MeOH)nm(ε); 280 (490), 271 (520).

Acetylation of heilonine; Heilonine (7.8mg) in acetic anhydride and pyridine (each 0.5ml) was heated 60°C in a water bath for 6h and allowed to stand at room temperature for overnight. The solution was evaporated under reduced pressure to dryness and yielded 8.4mg of diacetate.

Heilonine diacetate (2a); mp. 243-246°C., EI-MS (*m/z*); 493 (M^+), 478 (base peak), 396, 336, 179, 98., $[\alpha]_D$ +34° (*c* 1.05, $CHCl_3$)., IR $\upsilon_{max}(CHCl_3)$ cm^{-1}; 2850 (*trans*-indolidine), 1710 (carbonyl)., ^{1}H-NMR (270MHz, CDCl$_3$), ^{13}C-NMR (100MHz, CDCl$_3$); see Table 5.

References and Notes

1) Shen-nung-pen-tsao-ching (Shen-nung's Herbal); Chung-yao-chih (Chinese Herbal Drugs) (1979).
2) Y. Kitamura, M. Nishizawa, K. Kaneko, M. Ikura, K. Hikichi, M. Shiro, Y.-P. Chen, and H.-Y. Hsu, *Tetrahedron* , in Press.
3) Y. Kitamura, M. Nishizawa, K. Kaneko, M. Shiro, Y.-P. Chen, and H.-Y. Hsu, *Tetrahedron Lett.*, in Press.
4) The crystal of **1** belongs to the orthorhombic system with space group $p2_12_12_1$, and the cell dimentions a=15.064(6), b=17.546(4), c=11.178(3) Å^3, V=2954(1) Å^3, R=0.074 for 1769 reflections. The ring fusions in **1** are as follows; A/B *trans*, B/C *trans*, C/D *cis*, D/E *cis*, E/F *cis*. The configurations at the chiral centers have been settled as 3-OH β-equatorial, 12-OH α-axial, Me$_{19}$ β-axial, Me$_{21}$ α-axial, Me$_{27}$ β-equatorial, H-22 α-axial and the lone pair of nitrogen α-equatorial. Table of structural data are available from the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U. K.
5) The crystal of **2** belongs to the orthorhombic system with space group $p2_12_12_1$, and the cell dimentions a=13.185(1), b=14.340(1), c=12.268(1) Å^3, V=2319.5(4) Å^3, Z=4, D_C=1.173gcm^{-3}, μ=5.7cm^{-1}. Insentities of 2243 unique reflections in the region of $2\theta \leq 130$ were measured on a Rigaku diffractometer using Cu$K\alpha$ radiation. The structure was solved by direct methods and refined by block-diagonal least-squares technique to R=0.051 for 1922 reflections with $|Fo| > 3\sigma|Fo|$. The absolute configuration of the molecule shown in Fig. 3 was determined on the basis of the β-configuration of the Me$_{19}$ group. The ring fusions in 2 are as follows; A/B *trans*, B/C *trans*, E/F *trans*. The configurations at the chiral centers have been settled as 3-OH β-equatorial, 6-OH β-axial, Me$_{19}$ β-axial, Me$_{21}$ α-equatorial, Me$_{27}$ α-equatorial, H-22 α-axial and the lone pair of nitrogen β-axial. Table of structural data are available from the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road,Cambridge CB2 1EW, U. K.