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NEW STEROIDAL ALKALOIDS HAVING A NOVEL SEVEN RING SKELETON FROM FRITILLARIA USSURIENSIS MAXIM

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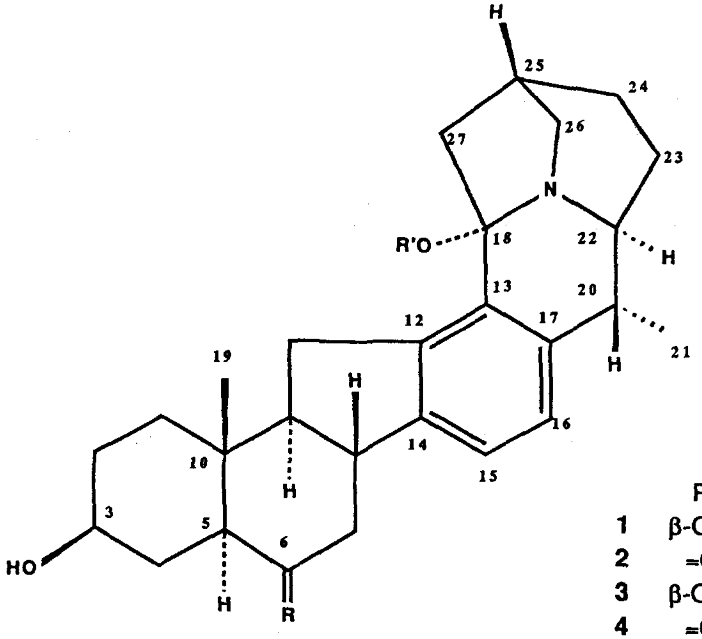
Abstract; Two new steroidal alkaloids having a novel seven ring skeleton, ussurienine (**1**) and ussurienone (**2**), were isolated from the HCl-MeOH hydrolyzed alkaloid fraction of *Fritillaria ussuriensis* Maxim. (Ping-bei-mu). Continuous study on the same plant, however, showed that **1** and **2** were the artifacts derived from ussuriedine (**3**) and ussuriedinone (**4**), which were isolated from the free alkaloids fraction. Their structures were elucidated on the basis of spectral data, X-ray crystal analysis and chemical conversion.

An important traditional Chinese medicine "Bei-mu", the dried bulbs of *Fritillaria* genus (Liliaceae), has been used as antitussive, sedative and expectorant in China.1) *Fritillaria* species are well known to contain cevane alkaloids; e.g. verticine,2) verticinone2) and imperialine.3) We have been studying chemical constituents of "Bei-mu", and isolated new cevane alkaloids; delavine,4) delavinone,4) delafrine,5) delafrinone,5) chuanbeinone6) and tortifoline.7) In the previous communication,8) we reported the isolation and structural elucidation of a new steroidal alkaloid having a novel skeleton, ussurienine (1), from the HCl-MeOH hydrolyzate of alkaloid containing

fraction of *Fritillaria ussuriensis* Maxim.
(Scheme). In this paper, we report the isolation and the structural elucidation of **1** and another new alkaloid, ussurienone (**2**), having the same skeleton. Moreover, our continuous study on *F. ussuriensis*, revealed that **1** and **2** were the artifacts produced on the acid hydrolysis in methanol during the isolation procedure. Also, we report two new alkaloids **3** and **4** which are the genuine alkaloids of **1** and **2**, respectively.

Results and Discussion

The dried bulbs of *F. ussuriensis* was extracted with 50% aqueous acetone, and the extract was fractionated by Diaion HP-20 column chromatography using H_2O-MeOH as a solvent system. The alkaloid-containing fraction (50~100% MeOH eluate) was hydrolyzed with 1N HCl-MeOH. The resulting crude alkaloid fraction was purified by repeated silica gel column chromatography. Two new alkaloids, ussurienine (**1**) and ussurienone (**2**), were isolated along with solanidine, verticine, verticinone and delavine.



	R	R'
1	β-OH	Me
2	=O	Me
3	β-OH	H
4	=O	H

Scheme

Ussurienine (**1**) was obtained as colorless needles from MeOH. The mass (MS) spectrum of **1** revealed the molecular ion (M^+) peak at *m/z* 437, and the base peak at *m/z* 406. The high resolution mass (HR-MS) spectrum of M^+ indicated the molecular formula of **1** as $C_{28}H_{39}NO_3$ (the index of hydrogen deficiency is 10). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, the signal of Me_{19} (δ 1.33) was observed lower field compared with that of 5α-cholestanol. This lower field shift was explained by the 1,3-diaxial interaction with the β-axial hydroxyl group at C6.[6] The other methyl signals were observed at δ1.38 (doublet, J =7.0 Hz) and δ 2.94 (singlet), two hydroxymethine signals at δ 3.64 ($W_{1/2}$=22 Hz) and 4.05 ($W_{1/2}$=10 Hz), and the aromatic proton signals at δ 6.86 and 7.05 (each, 1H, J = 7.6 Hz). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, four quaternary carbons and two methine carbons were observed in the aromatic carbon region. These data suggested the presence of a 1,2,3,4-tetrasubstituted aromatic ring in **1**(Table 1). The analysis of ^{1}H-^{1}H two-dimensional correlation spectroscopy (2D COSY) and ^{1}H-^{13}C 2D COSY spectra revealed the partial structures of **1**, and the connectivities around quaternary carbons (C12, C13, C14 and C17) were clarified by correlation spectroscopy *via* long range coupling (COLOC) technique (Fig. 1). Nuclear overhauser effect (NOE) experiment of **1** also confirmed these partial structures.

Finally, the structure of **1** was determined by X-ray crystallographic analysis, presuming Me_{19} is β-axial (Fig. 2). Compound **1** has a novel structure; the seventh additional ring formed by the linkage between C18-C27 of cevane skeleton and the aromatized D ring. The base peak (*m/z* 406) in the MS spectrum was explicable by elimination of the methoxyl group at C18.

Ussurienone (**2**) was obtained as a white amorphous powder. The MS spectrum of **2** revealed the M^+ peak at *m/z* 435 and the base peak at *m/z* 404 (M^+-OMe), and these were two mass unit smaller than those of **1**. Compound **2** showed a carbonyl absorption (1690 cm^{-1}) in the infrared (IR) spectrum, and a signal of carbonyl carbon was observed at δ 209.6 in the ^{13}C-NMR spectrum (Table 1). In the ^{1}H-NMR spectrum of **2**, there was only one signal of hydroxymethine proton at δ 3.71 ($W_{1/2}$=21 Hz) assignable

Table 1 ^{13}C-NMR data for 1, 2, 3 and 4 (C_5D_5N, δ)

Carbon No.	1	3	2	4
1	39.6 t	39.6	37.2	37.2 t
2	32.4 t	32.4	31.6	31.5 t
3	71.6 d	71.6	70.3	70.3 d
4	36.4 t	36.6	30.5	30.6 t
5	49.4 d	49.5	57.7	57.7 d
6	72.1 d	72.2	209.6	209.5 s
7	38.6 t	38.9	45.3	45.4 t
8	39.4 d	39.5	44.5	44.5 d
9	62.8 d	62.5*	61.2**	61.9 d***
10	36.3 s	36.3	38.7	38.6 s
11	31.2 t	31.4	31.1	31.0 t
12	146.4 s	146.4	146.4	144.1 s
13	139.5 s	137.4	139.5	138.0 s
14	142.7 s	143.4	142.7	142.5 s
15	121.6 d	121.3	121.9	121.2 d
16	127.3 d	127.0	127.3	127.3 d
17	131.8 s	136.9	131.8	137.2 s
18	97.8 s	92.4	97.7	92.2 s
19	15.9 q	15.9	13.8	13.8 q
20	40.1 d	40.4	40.2	40.3 d
21	22.5 q	22.6	22.4	22.4 q
22	61.6 d	62.1*	61.5**	60.9 d***
23	25.3 t	25.3	25.2	25.2 t
24	30.6 t	30.8	30.5	30.6 t
25	35.5 d	36.3	35.5	36.2 d
26	58.7 t	59.2	58.7	59.1 t
27	47.0 t	47.1	46.9	47.0 t
-OMe	50.2 q	——	50.1	——

*,**,*** : These assignments are interchangeable.

to 3α-H, and the signal of Me_{19} (δ 0.76) was observed at higher field than that of **1** (δ 1.33). All the other signal of ^{1}H- and ^{13}C-NMR spectra of **2** were resembled to those of **1**. Compound **2** was, therefore, deduced to be the 6-keto derivative of **1**. Reduction of **2** with sodium borohydride gave only one product which showed the same spectral data as those for **1**.

Ussurienine and ussurienone have a methoxyl group at C18 and this methoxyl group was thought to be introduced by the HCl-MeOH hydrolysis in the isolation procedure. This assumption was proved by the identification of C18-ethoxy derivative isolated from the HCl-EtOH treated alkaloid fraction [MS (*m/z*); 451 (M^+), 406 (base peak, M^+-OEt)]. We, therefore, attempted the isolation of alkaloids from which **1** and **2** were derived, using the procedure without HCl-MeOH hydrolysis.

The 50% aqueous acetone extract of the dried bulbs of *F. ussuriensis* was

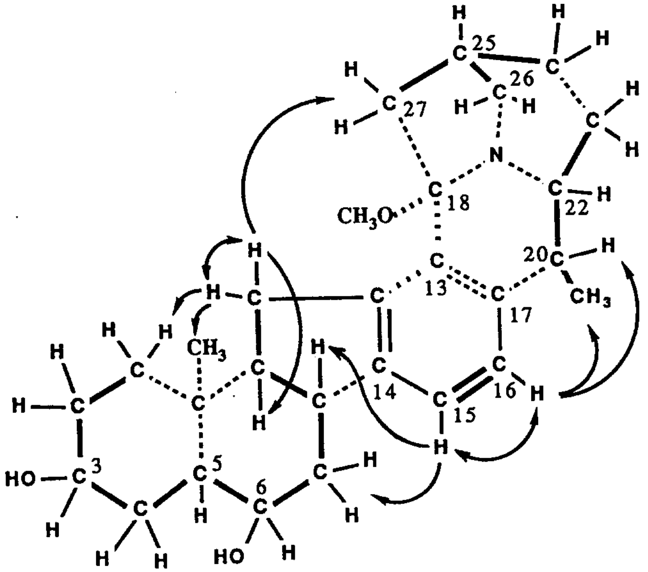
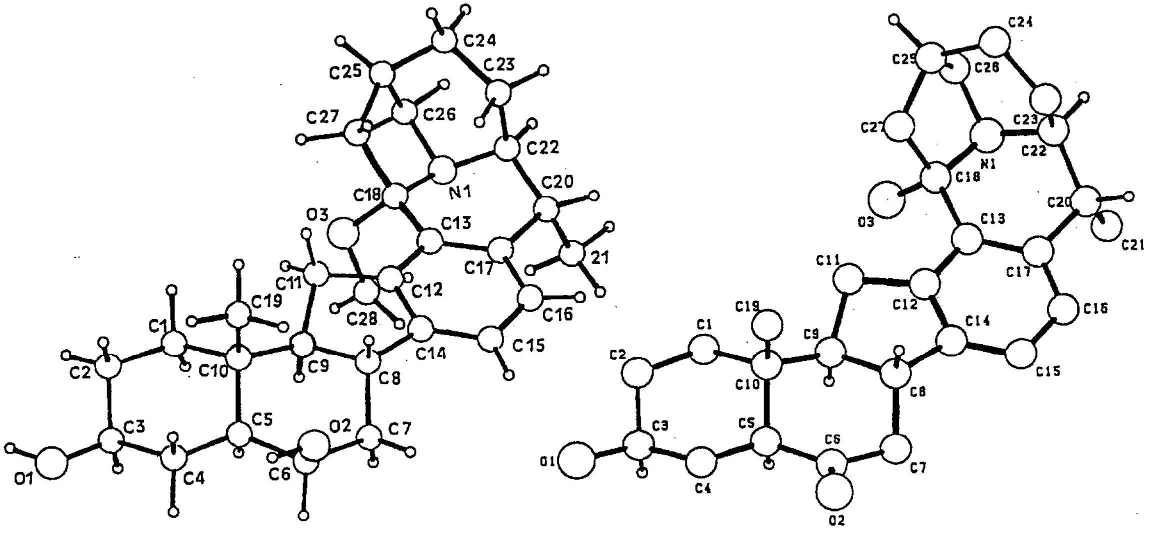


Fig. 1 The partial structures of **1**
Bold lines represent connectivities from 2D COSY experiments.
Plain lines represent connectivities from COLOC method.
Allows show NOE.



Ussurienine (1)

Ussuriedinone (4)

Fig. 2 Perspective Drawings of ussurienine (2) and ussuriedinone (4)

Table 2

Atomic coordinates (x10^4; x10^3 for H) and equivalent isotropic temperature factors (x10 $Å^{-2}$) with their e.s.d. values in parentheses, for ussurienine

	x	y	z	B_{eq}
C(1)	809(3)	9116(1)	5624(4)	29(1)
C(2)	-287(3)	9255(1)	6692(4)	29(1)
C(3)	-674(3)	9787(1)	6344(4)	29(1)
C(4)	-970(3)	9859(1)	4529(4)	29(1)
C(5)	137(3)	9716(1)	3470(4)	24(1)
C(6)	46(3)	9873(1)	1635(4)	29(1)
C(7)	1190(3)	9733(1)	652(4)	29(1)
C(8)	1565(3)	9192(1)	958(4)	25(1)
C(9)	1745(3)	9114(1)	2804(4)	25(1)
C(10)	540(3)	9168(1)	3766(4)	22(1)
C(11)	2527(3)	8640(1)	2938(4)	28(1)
C(12)	3314(3)	8681(1)	1416(4)	27(1)
C(13)	4445(3)	8451(1)	1061(4)	29(1)
C(14)	2754(3)	8986(1)	251(4)	28(1)
C(15)	3254(3)	9046(1)	-1305(4)	34(1)
C(16)	4327(3)	8794(1)	-1696(5)	38(1)
C(17)	4940(3)	8503(1)	-517(5)	34(1)
C(18)	5140(3)	8185(1)	2421(5)	33(1)
C(19)	-425(3)	8786(1)	3239(5)	31(1)
C(20)	6125(4)	8247(2)	-1006(5)	46(2)
C(21)	7179(4)	8629(2)	-1105(8)	72(2)
C(22)	6423(4)	7838(1)	226(6)	47(2)
C(23)	5568(5)	7383(2)	-8(7)	60(2)
C(24)	5628(5)	7034(2)	1489(8)	73(2)
C(25)	5607(4)	7327(2)	3103(7)	60(2)
C(26)	6736(4)	7662(2)	3138(7)	59(2)
C(27)	4545(4)	7703(1)	3120(6)	47(2)
C(28)	5856(4)	8961(2)	3530(6)	53(2)
N(1)	6391(3)	8043(1)	1908(4)	41(1)
O(1)	-1743(2)	9919(1)	7277(3)	38(1)
O(2)	-1023(2)	9679(1)	808(3)	34(1)
O(3)	5251(2)	8500(1)	3832(3)	40(1)
H(C1)	113(4)	875(1)	590(5)	29
H'(C1)	156(4)	933(1)	576(5)	29
H(C2)	-1(4)	922(1)	796(5)	29
H'(C2)	-103(4)	903(1)	640(5)	29
H(C3)	8(4)	1004(1)	660(5)	29
H(C4)	-119(4)	1024(1)	437(5)	29
H'(C4)	-171(4)	966(1)	414(5)	29
H(C5)	87(4)	990(1)	391(5)	24
H(C6)	-7(4)	1027(1)	160(5)	29
H(C7)	99(4)	980(1)	-58(5)	29
H'(C7)	191(3)	997(1)	94(5)	29
H(C8)	87(4)	899(1)	54(5)	25
H(C9)	233(4)	940(1)	320(5)	25
H(C11)	300(4)	863(1)	404(5)	28
H'(C11)	203(4)	831(1)	294(5)	28
H(C15)	279(4)	928(1)	-209(5)	34
H(C16)	461(4)	878(1)	-284(6)	38
H(C19)	-64(4)	884(1)	193(5)	31
H'(C19)	-10(4)	844(1)	346(5)	31
H"(C19)	-125(4)	881(1)	397(5)	31
H(C20)	606(5)	811(2)	-229(6)	46
H(C21)	795(5)	846(2)	-161(7)	72
H'(C21)	735(5)	879(2)	9(7)	72
H"(C21)	688(5)	888(2)	-205(7)	72
H(C22)	727(5)	770(1)	7(6)	47
H(C23)	457(5)	755(2)	-11(7)	60
H'(C23)	583(5)	721(2)	-104(7)	60
H(C24)	490(5)	680(2)	142(7)	73
H'(C24)	648(5)	685(2)	145(7)	73
H(C25)	567(5)	711(2)	426(7)	60
H(C26)	753(5)	749(2)	258(6)	60
H'(C26)	687(5)	785(2)	430(7)	60
H(C27)	427(5)	777(2)	445(6)	47
H'(C27)	378(4)	759(2)	240(6)	47
H(C28)	611(4)	912(2)	452(6)	53
H'(C28)	568(5)	916(2)	252(6)	53
H"(C28)	673(4)	889(2)	295(6)	53
H(O1)	-155(4)	985(1)	849(6)	38
H(O2)	-177(4)	975(1)	148(5)	34

Table 3 Anisotropic thermal parameters (x10^4) with their e.s.d. values in parentheses, for ussurienine

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	57(3)	9(1)	122(6)	4(1)	4(4)	3(1)
C(2)	66(3)	12(1)	85(5)	4(1)	7(4)	0(1)
C(3)	59(3)	11(1)	106(6)	7(1)	2(3)	-3(1)
C(4)	60(3)	11(1)	96(5)	7(1)	-6(3)	0(1)
C(5)	49(3)	8(1)	97(5)	2(1)	4(3)	0(1)
C(6)	59(3)	11(1)	109(5)	4(1)	-16(3)	4(1)
C(7)	63(3)	12(1)	88(5)	2(1)	5(3)	6(1)
C(8)	46(3)	10(1)	85(5)	1(1)	6(3)	4(1)
C(9)	51(3)	8(1)	95(5)	0(1)	3(3)	0(1)
C(10)	45(3)	8(1)	72(5)	2(1)	-5(3)	-1(1)
C(11)	57(3)	11(1)	101(5)	4(1)	9(3)	2(1)
C(12)	57(3)	9(1)	101(5)	0(1)	5(3)	0(1)
C(13)	49(3)	9(1)	137(6)	0(1)	17(4)	1(1)
C(14)	55(3)	11(1)	102(5)	-1(1)	2(3)	0(1)
C(15)	60(3)	14(1)	123(6)	-1(1)	6(4)	2(1)
C(16)	72(3)	17(1)	120(6)	0(1)	18(4)	-1(1)
C(17)	63(3)	12(1)	141(6)	0(1)	21(4)	-1(1)
C(18)	57(3)	10(1)	165(7)	6(1)	13(4)	2(1)
C(19)	62(3)	10(1)	125(6)	0(1)	8(4)	-5(1)
C(20)	81(4)	17(1)	189(8)	9(1)	49(5)	2(2)
C(21)	72(4)	23(1)	436(16)	4(1)	64(7)	37(3)
C(22)	81(4)	14(1)	239(9)	10(1)	42(5)	3(2)
C(23)	134(6)	13(1)	308(11)	-1(1)	60(7)	-20(2)
C(24)	144(6)	11(1)	447(16)	6(1)	77(9)	7(3)
C(25)	118(5)	14(1)	319(12)	13(1)	54(7)	19(2)
C(26)	97(5)	17(1)	313(11)	18(1)	23(7)	21(2)
C(27)	92(4)	10(1)	255(9)	10(1)	36(5)	17(2)
C(28)	110(5)	16(1)	238(9)	-7(1)	-16(6)	-7(2)
N(1)	59(3)	14(1)	211(7)	6(1)	28(4)	5(1)
O(1)	75(2)	17(1)	107(4)	14(1)	10(2)	-2(1)
O(2)	59(2)	17(1)	99(4)	5(1)	-5(2)	-4(1)
O(3)	80(3)	15(1)	147(4)	7(1)	-10(3)	1(1)

The temperature factor is of the form:

$$\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)].$$

Table 4

Bond lengths (Å) with their e.s.d. values in parentheses, for ussurienine

C(1)	- C(2)	1.520(5)	C(1)	- C(10)	1.540(5)
C(2)	- C(3)	1.522(5)	C(3)	- C(4)	1.517(5)
C(3)	- O(1)	1.432(4)	C(4)	- C(5)	1.528(5)
C(5)	- C(6)	1.549(5)	C(5)	- C(10)	1.561(5)
C(6)	- C(7)	1.525(5)	C(6)	- O(2)	1.440(4)
C(7)	- C(8)	1.536(5)	C(8)	- C(9)	1.523(5)
C(8)	- C(14)	1.520(5)	C(9)	- C(10)	1.532(5)
C(9)	- C(11)	1.540(5)	C(10)	- C(19)	1.532(5)
C(11)	- C(12)	1.505(5)	C(12)	- C(13)	1.408(5)
C(12)	- C(14)	1.392(5)	C(13)	- C(17)	1.394(5)
C(13)	- C(18)	1.517(5)	C(14)	- C(15)	1.382(5)
C(15)	- C(16)	1.387(5)	C(16)	- C(17)	1.405(6)
C(17)	- C(20)	1.515(7)	C(18)	- C(27)	1.559(6)
C(18)	- N(1)	1.473(5)	C(18)	- O(3)	1.429(5)
C(20)	- C(21)	1.544(8)	C(20)	- C(22)	1.523(7)
C(22)	- C(23)	1.552(7)	C(22)	- N(1)	1.471(6)
C(23)	- C(24)	1.537(9)	C(24)	- C(25)	1.528(9)
C(25)	- C(26)	1.525(8)	C(25)	- C(27)	1.537(7)
C(26)	- N(1)	1.480(7)	C(28)	- O(3)	1.428(6)

Table 5 Bond angles (°) with their e.s.d. values in parentheses, for ussurienine

C(2) -C(1) -C(10)	112,6(3)	C(1) -C(2) -C(3)	110.1(3)
C(2) -C(3) -C(4)	111.0(3)	C(2) -C(3) -O(1)	111.2(3)
C(4) -C(3) -O(1)	107.9(3)	C(3) -C(4) -C(5)	110.1(3)
C(4) -C(5) -C(6)	114.8(3)	C(4) -C(5) -C(10)	112.0(3)
C(6) -C(5) -C(10)	115.1(3)	C(5) -C(6) -C(7)	112.4(3)
C(5) -C(6) -O(2)	113.5(3)	C(7) -C(6) -O(2)	109.0(3)
C(6) -C(7) -C(8)	111.6(3)	C(7) -C(8) -C(9)	108.9(3)
C(7) -C(8) -C(14)	120.9(3)	C(9) -C(8) -C(14)	102.1(3)
C(8) -C(9) -C(10)	112.1(3)	C(8) -C(9) -C(11)	104.8(3)
C(10)-C(9) -C(11)	121.1(3)	C(1) -C(10)-C(5)	106.9(3)
C(1) -C(10)-C(9)	109.0(3)	C(1) -C(10)-C(19)	110.0(3)
C(5) -C(10)-C(9)	104.6(3)	C(5) -C(10)-C(19)	113.7(3)
C(9) -C(10)-C(19)	112.4(3)	C(9) -C(11)-C(12)	101.3(3)
C(11)-C(12)-C(13)	129.2(3)	C(11)-C(12)-C(14)	110.5(3)
C(13)-C(12)-C(14)	120.3(3)	C(12)-C(13)-C(17)	118.7(3)
C(12)-C(13)-C(18)	119.7(3)	C(17)-C(13)-C(18)	121.4(3)
C(8) -C(14)-C(12)	109.5(3)	C(8) -C(14)-C(15)	129.5(3)
C(12)-C(14)-C(15)	121.0(3)	C(14)-C(15)-C(16)	118.8(3)
C(15)-C(16)-C(17)	121.2(4)	C(13)-C(17)-C(16)	119.8(4)
C(13)-C(17)-C(20)	121.5(4)	C(16)-C(17)-C(20)	118.7(4)
C(13)-C(18)-C(27)	116.8(3)	C(13)-C(18)-N(1)	112.2(3)
C(13)-C(18)-O(3)	110.0(3)	C(27)-C(18)-N(1)	105.6(3)
C(27)-C(18)-O(3)	103.9(3)	N(1) -C(18)-O(3)	107.6(3)
C(17)-C(20)-C(21)	109.9(4)	C(17)-C(20)-C(22)	109.9(4)
C(21)-C(20)-C(22)	111.1(4)	C(20)-C(22)-C(23)	111.5(4)
C(20)-C(22)-N(1)	109.2(4)	C(23)-C(22)-N(1)	113.4(4)
C(22)-C(23)-C(24)	111.3(5)	C(23)-C(24)-C(25)	110.9(5)
C(24)-C(25)-C(26)	108.1(5)	C(24)-C(25)-C(27)	111.1(5)
C(26)-C(25)-C(27)	102.3(4)	C(25)-C(26)-N(1)	101.2(4)
C(18)-C(27)-C(25)	103.6(4)	C(18)-N(1) -C(22)	112.4(3)
C(18)-N(1) -C(26)	103.0(3)	C(22)-N(1) -C(26)	110.9(4)
C(18)-O(3) -C(28)	114.8(3)		

fractionated as mentioned above. The alkaloid-containing fraction was dissolved in 0.05N HCl, and was washed with ethyl acetate to remove fatty substances. The aqueous solution was made alkaline with NH_4OH, and was partitioned with ether and butanol, successively. The ether soluble portion, which contained free alkaloids, was fractionated by repeated silica gel column chromatography, and two new steroidal alkaloids (**3** and **4**) were obtained, along with solanidine, verticine and peimisine.[10)]

Ussuriedine (**3**) was obtained as a white amorphous. In the MS spectrum of **3**, the M^+ peak was observed at *m/z* 423 and the base peak at *m/z* 390. The HR-MS spectrum of M^+ indicated the molecular formula of **3** as $C_{27}H_{37}NO_3$ (the index of hydrogen deficiency is also 10). In the ^{1}H-NMR spectrum, **3** showed a tertiary and a secondary methyl signals (δ 1.37 and δ 1.29, *J* =7.3 Hz, respectively), and two aromatic protons coupled each other (δ 6.83, δ 7.03, each *J* =7.3 Hz). In the ^{13}C-NMR spectrum, the signals of two CH_3, nine CH_2, nine CH and seven quaternary carbons were observed, and two of methine carbons and four of quaternary carbons were in aromatic carbon region (Table 1). These observations suggested that **3** has the same skeleton as **1**. In the ^{1}H-NMR spectrum, however, **3** showed no methoxy signal, and there was no M^+-OMe ion peak in the MS spectrum. Moreover, in the ^{13}C-NMR spectrum of **3**, there was no methoxy signal, and the signal of C18 (δ 92.4) was observed at higher field than that of **1** (δ 97.8).

On the basis of above results, **3** was assumed to be the C18-hydroxy derivative of **1**. This assumption was proved by the conversion of **3** to **1** on the treatment with MeOH. Compound **1** was shown to be the artifact derived from **3** on methanolic HCl hydrolysis during the isolation procedure.

Ussuriedinone (**4**) was obtained as colorless needles from acetone. The MS spectrum of **4** showed M^+ and the base peak at *m/z* 421 and 388, respectively, which were two mass unit smaller than those of **3**. Compound **4** was deduced to be the 6-keto derivative of **3** from the results of IR (1700 cm^{-1}), ^1H-NMR (δ 0.76, Me_{19}), and ^{13}C-NMR (δ 209.5, C6) spectra (Table 1). Reduction of **4** with sodium borohydride gave only one product, which showed the same spectral data as those for **3**. Finally, the absolute configuration of **4** was proved by X-ray crystallographic analysis,[11] presuming Me_{19} is β-axial (Fig. 2).

Compound **4** was also converted to its C18-OMe derivative (**2**) on the treatment withmethanolic HCl. Compound **2** was shown to be the artifact derived from **4** on methanolic HCl hydrolysis during the isolation procedure.

On the other hand, the butanol soluble portion was assumed to contain alkaloid glycosides, and was treated with β-glucosidase. The obtained aglycone fraction was fractionated by silica gel column chromatography to give **3** and **4**. So, **3** and **4** are also present as their glucosides in *F. ussuriensis.*

Compound **1**, **2**, **3** and **4** have a seven ring skeleton; in addition to cevane skeleton, the C-C bond between C18 and C27 forms G ring, -C18-N-C26-C25-C27. The E, F, and G rings constitute a tricyclo-[4.4.0.$1^{4,7}$]-6-aza-undecane partial structure. And the Dreiding model consideration showed that this tricyclo structure has little strain. It is required to form the tricyclo ring sysytem that the E and F ring has a *cis* -quinolizidine structure, in which the lone pair of nitrogen and H-22 are both in α-configuration.

According to the knowledge of the biosynthesis of cevane alkaloids,[12] C18 and C27 are not active sites and are located far from each other. The carbon-carbon bond formation between C18 and C27 is, therefore, a unique example in cevane alkaloid biosynthesis. The elucidatiom of the biosynthesis of **3** and **4** must be important in the field of steroidal alkaloid biosynthesis.

The high performance liquid chromatography (HPLC) analysis[13)] showed that ussuriedine (**3**) and ussuriedinone (**4**) are the characteristic alkaloids of *F. ussuriensis.* It is also important to know the chemotaxonomic relation between *F. ussuriensis* and other *Fritillaria* species.

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Experimental

Melting points were determined on a Leitz Wetzlar micro melting-point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 spectrometer. ^{1}H-NMR spectra were run on a JEOL JNM FX-100 (100 MHz), GX-270 (270 MHz), or GX-500 (500 MHz) spectrometer in C_5D_5N solution, and ^{13}C-NMR spectra on a JEOL FX-90Q (22.5 MHz), GX-270 (67.5 MHz), or GX-500 (125 MHz) spectrometer in C_5D_5N solution. Electron impact mass spectrometry (EI-MS) was measured with a JEOL JMS D-300 or a DX-303 spectrometer. UV spectra was measured with a Shimadzu UV-220 spectrometer. IR spectra was measured with a JASCO A-102 spectrometer. Thin-layer chromatography (TLC) was performed on Merck precoated Kiesel gel 60 F_{254} plates. Column chromatography was carried out on Wakogel C-300, Kiesel gel 60, and Diaion HP-20 (Mitsubishi Kasei Co., Ltd.).

Isolation of 1 and 2: The dried bulbs of *F.ussuriensis* (14 kg, obtained in Harbin market, China), were powdered and extracted with 50% aqueous acetone (15 lx4). After concentration to 5 l, the aqueous solution was washed with hexane (2 l) to remove fatty substances (28.3 g), and then applied to a column (9 x 90 cm) of Diaion HP-20. The column was eluted with H_2O-MeOH increasing MeOH concentration 0% to 100% stepwisely. The alkaloid-containing fractions (50-100% MeOH eluate) were combined and concentrated (42.5 g). The combined alkaloid-containing fraction was hydrolyzed with 1N HCl-MeOH (650 ml) for 6 h, and worked up to afford 9.7 g of crude alkaloid fraction. The crude alkaloid was fractionated by repeated silica gel column chromatography using the solvent systems of hexane-EtOH-Et_2NH (12:1:1) and hexane-EtOAc-MeOH (2:2:1). Ussurienine (**1**, 204.1 mg) and ussurienone (**2**, 10.9 mg) were obtained along with solanidine (48.4 mg), verticine (15.7 mg), verticinone (4.5 mg), and delavine (1.2 mg).

Ussurienine (**1**): mp. >300°C., HR-MS; $C_{28}H_{39}NO_3$ found: 437.2928, calcd.: 437.2930., EI-MS (*m/z*); 437 (M^+), 422, 406 (M^+-OCH_3, base peak), 390, 362., $[\alpha]_D$ +20° (*c* 0.92, $CHCl_3$)., IR $\nu_{max}(CHCl_3)cm^{-1}$; 3450(OH)., UV λ_{max}(MeOH)nm(ε); 280(1300), 271(1300)., ^{1}H-NMR (500 MHz, C_5D_5N), δ; 1.33 (3H, s, 19-H), 1.38 (3H, d, J =7.0 Hz, 21-H), 2.94 (3H, s, OMe), 3.64 (1H, m, $W_{1/2}$=22 Hz, 3α-H), 4.05 (1H, m, $W_{1/2}$=10 Hz, 6α-H), 6.86 (1H, d, J =7.6 Hz, 16-H), 7.05 (1H, d, J =7.6 Hz, 15-H)., ^{13}C-NMR (100 MHz, C_5D_5N); see Table 1.

Ussurienone (2): mp. 110-116°C., HR-MS; $C_{28}H_{37}NO_3$ found: 435.2789, calcd.: 435.2773., EI-MS (*m/z*); 435 (M^+), 420, 404 (M^+-OCH_3, base peak), 388., $[\alpha]_D$ +8° (*c* 0.71, $CHCl_3$)., IR υ_{max}($CHCl_3$)cm^{-1}; 3350(OH), 1690 (carbonyl)., UV λ_{max}(MeOH)nm(ε); 281(1400), 272(1600)., ^{1}H-NMR (400 MHz, C_5D_5N), δ; 0.76 (3H, s, 19-H), 1.40 (3H, d, J =7.1 Hz, 21-H), 2.97 (3H, s, OMe), 3.71 (1H, m, $W_{1/2}$=21 Hz, 3α-H), 6.87 (1H, d, J =7.6 Hz, 16-H), 6.95 (1H, d, J=7.6 Hz, 15-H)., ^{13}C-NMR (22.5 MHz, C_5D_5N); see Table 1.

Reduction of ussurienone (2) with $NaBH_4$ to ussurienine (1): To a solution of **2** (10.4 mg) in EtOH (5 ml), excess $NaBH_4$ was added and the whole mixture was stirred for 1 h at room temparature. After addition of cool water, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated to dryness. Purification by column chromatography yielded 6.5 mg of **1**, the product showed same Rf value as ussurienine on TLC and same MS spectrum.

Isolation of 3 and 4: The dried bulbs of *F. ussuriensis* (30 kg) were powdered and extracted with hexane-ether (1:1, 18 lx4) and then extracted with 50% aqueous acetone (18 lx6). The 50% aqueous acetone extract was concentrated to 5 l, and was applied to a column (9 x 90cm) of Diaion HP-20. The column was eluted with H_2O-MeOH. The alkaloid-containing fractions were combined and concentrated (151.3 g). The alkaloid-containing fraction was dissolved in 0.05N HCl (3 l), and washed with ethyl acetate (1 l) to remove fatty acid. The aqueous solution was made alkaline with NH_4OH, and was partitioned with ether (1 lx6) and butanol (1 lx6), successively. The ether soluble fraction was extracted with 5% tartaric acid (2 l), and the 5% tartaric acid layer was made alkaline and was extracted with $CHCl_3$ (600 mlx3). The $CHCl_3$ layer was evaporated under reduced pressure, and 13.5 g of free alkaloids fraction was obtained. The free alkaloids fraction was fractionated repeatedly silica gel column chromatagraphy using same solvent systems as mentioned above. Ussuriedine (**3**, 88.8 mg) and ussuriedinone (**4**, 121.7 mg) were obtained along with solanidine (4.9 mg), verticinone (38.8 mg) and peimisine (210.0 mg).

The butanol soluble fraction was washed with 5% tartaric acid (2 l), and the 5% tartaric acid layer was made alkaline and was extracted with butanol (600 mlx3). The butanol layer was evaporated to dryness under reduced pressure and yielded 22.5 g of alkaloid glycoside fraction. The alkaloid glycoside fraction was dissolved in 2.3 l of acetate buffer (pH 5.5), and β-glucosidase (23 g, snail acetone powder, Sigma S-9764) was added to the solution. The mixture was allowed to stand for 66 h at 25°C. After addition of NH_4OH, aglycone (5.8 g) was isolated by the same method as mentioned above. The aglycones fraction was fractionated repeatedly by silica gel column chromatagraphy using same solvent sysytems as mentioned above to yield **3** and **4**.

Ussuriedine (3): mp. 189.5-192.5°C., HR-MS; $C_{27}H_{37}NO_3$ found: 423.2764, calcd.: 423.2773., EI-MS (*m/z*); 423 (M^+), 408, 390(base peak), 362., $[\alpha]_D$ +19° (*c* 0.79, $CHCl_3$)., IR $\nu_{max}(CHCl_3)cm^{-1}$; 3400(OH)., UV $\lambda_{max}(MeOH)nm(\varepsilon)$; 281(1300), 272(1100)., ^{1}H-NMR (500 MHz, C_5D_5N), δ; 1.29 (3H, d, *J* =7.3 Hz, 21-H), 1.37 (3H, s, 19-H), 3.78 (1H, m, $W_{1/2}$=24 Hz, 3α-H), 4.05 (1H, m, $W_{1/2}$=6 Hz, 6α-H), 6.83 (1H, d, *J* =7.3 Hz, 16-H), 7.03 (1H, d, *J* =7.3 Hz, 15-H)., ^{13}C-NMR (22.5 MHz, C_5D_5N); see Table 1.

Ussuriedinone (4): mp. 268-272°C., HR-MS; $C_{27}H_{35}NO_3$ found: 421.2605, calcd.: 421.2617., EI-MS (*m/z*); 421 (M^+, base peak), 406, 388, 360., $[\alpha]_D$ +12° (*c* 0.80, $CHCl_3$)., IR $\nu_{max}(CHCl_3)cm^{-1}$; 3400(OH), 1700 (carbonyl)., UV $\lambda_{max}(MeOH)nm(\varepsilon)$; 281(1600), 272(1500)., ^{1}H-NMR (270 MHz, C_5D_5N), δ; 0.76 (3H, s, 19-H), 1.29 (3H, d, *J* =7.0 Hz, 21-H), 3.62 (1H, m, $W_{1/2}$=23 Hz, 3α-H), 6.84 (1H, d, *J* =7.7 Hz, 16-H), 6.92 (1H, d, *J* =7.7 Hz, 15-H)., ^{13}C-NMR (22.5 MHz, C_5D_5N); see Table 1.

Reduction of ussuriedinone (4) with $NaBH_4$ to ussuriedine (3): To a solution of **4** (11.6 mg) in MeOH (5 ml), excess $NaBH_4$ was added and the whole mixture was stirred for 2 h at room temparature. After addition of cool water, the reaction mixture was worked up by above method. Purification by column chromatography yielded 6.1 mg of **3**, the product showed same Rf value as ussuriedine on TLC and same MS spectrum.

Conversion of 3 to 1: Compound **3** in 1N HCl-MeOH was heated in a water bath for 1h. The solution was made alkaline, and extracted with $CHCl_3$. The product showed same Rf valueas **1** on TLC. Compound **4** was also converted to **2** by the same method.

References and Notes

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9) The crystal of **1** belongs to the orthorhombic system with space group $P2_12_12_1$, and the cell dimentions a=10.877 (2), b=26.983 (3), c=8.100 (1) Å, V=2377.1 (5) Å^3, Z=4,

D_C=1.222gcm^{-3}. Insentities of 1748 unique reflections in the region of $2\theta \leq 110°$ 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=0.038 for1544 reflections with $|F_o| > 3\sigma(F_o)$. The absolute configuration of the molecule shown in Fig. 2was determined on the basis of the β-configuration of the Me_{19} group.

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13)The crude alkaloid fractions, obtained by acid hydrolysis from 19 species of *Fritillaria* genus (dried bulbs), were treated with 3,4-dichlorophenyl isocyanate in pyridine and toluene. The resulted carbamates were analyzed by HPLC. Ussurienine was detected only in *F. ussuriensis* (unpublished data, 19 *Fritillaria* genus were supplied from Prof. G.-J. Xu).