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USSURIENINE, A NOVEL 5α-CEVANINE ALKALOID FROM FRITILLARIA USSURIENSIS MAXIM.

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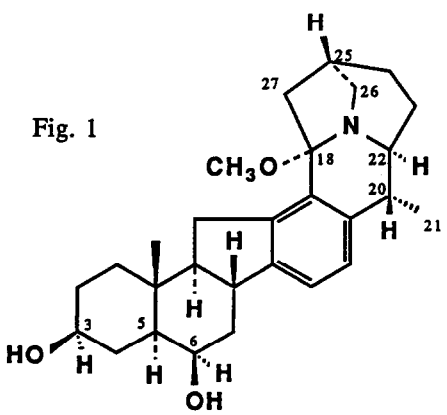
SUMMARY: The structure of ussurienine, a novel 5α-cevanine alkaloid isolated from *Fritillaria ussuriensis* has been deduced as 1 from its NMR studies and confirmed by X-ray crystallographyic analysis.

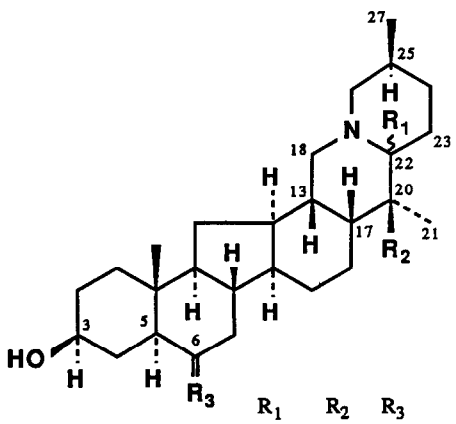
"Bei-mu", the bulbs of the Fritillaria genus (Liliacea), have been used as an antitussive and expectrant, and for other proposes, is an important traditional Chinese medicine.[1] In the main land China, there are many kinds of bei-mu, produced from different species of the Fritillaria genus in several provinces.

In our project of the establishment of the relationships between the structure of constituents and their pharmacological effects among the several kinds of "bei-mu", we have previously reported the structures of delavine(2), delavinone(3), and chuanbeinone(4) from *Fritillaria delavayi* Franch, "chuan-bei-mu".[2),3)] In this paper, we describe the structural elucidation of a new novel Fritillaria alkaloid, ussurienine(1), isolated from *F. ussuriensis* ("ping-bei-mu").

Fig. 1



Ussurienine (1)



	R_1	R_2	R_3
Delavine (2)	α -H	H	β-OH
Delavinone (3)	α -H	H	=O
Chuanbeinone(4)	β -H	H	=O

The dried powder of *F.ussuriensis* was extracted with 50% acetone, and the extract was hydrolyzed with 1N HCl-MeOH. The resulting crude alkaloids were purified by the silica gel column chromatography. One of the main alkaloid, ussurienine(1, 0.024%) was isolated, in addition to solanidine, verticinone, and imperialine as minor alkaloids.

Ussurienine(1) was crystallized from MeOH, colorless needle, and slightly soluble in chloroform, methanol, and acetone. Other physicochemical properties were shown in Table 1. In the ^{1}H-NMR spectrum of 1 (Py-d_5), the tertial methyl group at δ 1.57(19-H) was shifted downfield, compared with that of 5α-cholestanol, because of the 1,3-diaxial interaction with the β-axial hydroxyl group at C-6. Another methyl signals showed at δ 1.44(doublet, J=7.3Hz), δ 2.90(singlet). Two signals at δ 3.72($W_{1/2}$=21Hz) and 4.03($W_{1/2}$=8Hz) were assigned to methine protons bearing oxygen function, these signals shifted downfield to δ 5.00 and 5.19 on acetylation, respectively. The aromatic proton signals at δ 6.85 and 7.08 (each, 1H, J=7Hz) and six sp^2 carbon signals in the ^{13}C-NMR of 1, was suggested the appearance of 1,2,3,4-tetrasubstituted aromatic ring in 1. The structure analysis of 1 by ^{1}H-^{1}H 2D COSY NMR and ^{1}H-^{13}C 2D COSY NMR spectra were allowed to assign the most of the ^{1}H and ^{13}C signals of ussurienine(1) (Table 1 and Fig.2). The results of NOE correlations spectrum was illustrated by allow line in Fig.2, and the results also supported the structure of 1. In the ^{13}C-NMR spectrum of 1, each carbon was tentatively assigned except the following carbons. The assignment of sp^2 carbons (C-12, C-13, C-14 , C-17) elucidated by COLOC (correlation spectroscopy *via* long range coupling) technique. The extra signal at δ 97.8 was compatible with the presence of CH_3O-C-N in 1, because this signal shifted downfield, compared with that of

Table 1 Physicochemical properties of 1

mp. 300°C, $[\alpha]_D$ +19.7° ($CHCl_3$, c=0.92)
MS; *m/z* 437(M^+), 422(M^+-Me), 406(M^+-OMe, base peak), 390, 362
HR-MS; $C_{28}H_{39}NO_3$, (Found: 437.29280, Calcd: 437.29295)
UV; λ max (MeOH, nm), 280 (ε, 1280), 271 (ε, 1280)
IR; ν max ($CHCl_3$, cm^{-1}) , 3450(OH), 2820(*trans* -quinolizidine)
^{1}H-NMR(δ, ppm, Py-d5); 1.44(3H, *d*, J=7.3Hz), 1.57(1H, *s*, 19-H), 2.90(3H, *s*, OMe)
3.72(1H, *m*, $W_{1/2}$=21Hz), 4.03(1H, *m*, $W_{1/2}$=8Hz)
6.85(1H, aromatic), 7.08(1H, aromatic)
^{13} C-NMR(δ, ppm, Py-d5); 39.6(*t*, C-1), 32.4(*t*, C-2), 71.6(*d*, C-3), 36.4(*t*, C-4), 49.4(*d*, C-5),
72.1(*d*, C-6), 38.6(*t*, C-7), 40.1(*d*, C-8), 62.8(*d*, C-9),
36.3(*s*, C-10),31.2(*t*, C-11), 139.5(*s*, C-12), 131.8(*s*, C-13),
146.4(*s*, C-14), 127.3(*d*, C-15), 121.6(*d*, C-16), 142.7(*s*, C-17),
97.8(*s*, C-18), 15.9(*q*, C-19), 39.4(*d*, C-20), 22.5(*q*, C-21),
61.6(*d*, C-22), 25.3(*t*, C-23), 30.6(*t*, C-24), 35.5(*d*, C-25),
58.7(*t*, C-26), 47.0(*t*, C-27), 50.2(*q*, OCH_3)

methoxyl carbon. Also in the MS spectrum of 1, the base peak at *m/z* 406 corresponded to loss of methoxyl groups. The proton at δ 2.90 in the ^{1}H-NMR spectrum in 1 was assigned as methoxyl protons, this signal shifted upfield by the shielding effect from the aromatic ring and by the electrophilicity of nitrogen. Also the secondary methyl signal shifted downfield to δ 1.44 by the deshielding effect from aromatic ring. From this reason, the methyl signal at δ 1.44 can assign to the methyl group at C-20.

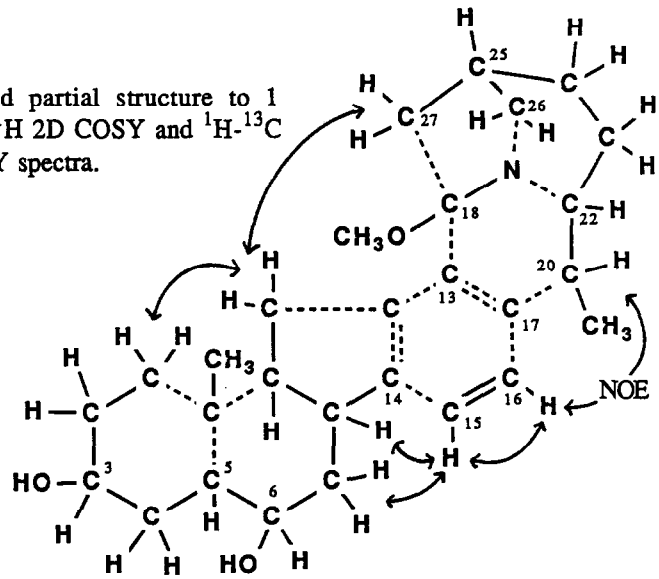


Fig. 2
Suggested partial structure to 1 from ^{1}H-^{1}H 2D COSY and ^{1}H-^{13}C 2D COSY spectra.

To resolute such obscurity in the structure of 1, especially the lack of the methyl group at C-25, we examined the X-ray crystal structure elucidation.

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^3, 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 $|Fo| > 3\sigma(Fo)$. The absolute configuration of the molecule shown in Fig.3 was determined on the basis of the β-configuration of the C-10 methyl group. The ring fusions in 1 are as follows; A/B *trans*, B/C *trans*, D aromatic, E/F *cis*. The configurations at the chiral centers have been settled as 3-OH β-equatorial, 10-Me β-axial, 20-Me α-axial, 22-H α-axial and the lone pair of nitrogen β-axial. The six membered ring A, B, E, F are in the chair configuration. The X-ray crystal structure of 1 illustrated a quite novel structure, namely, the additional ring structure by the binding between C-18 and C-27, and the D ring being aromatized on the 5α-cevanine-3β, 6β-diol carbon skeleton.

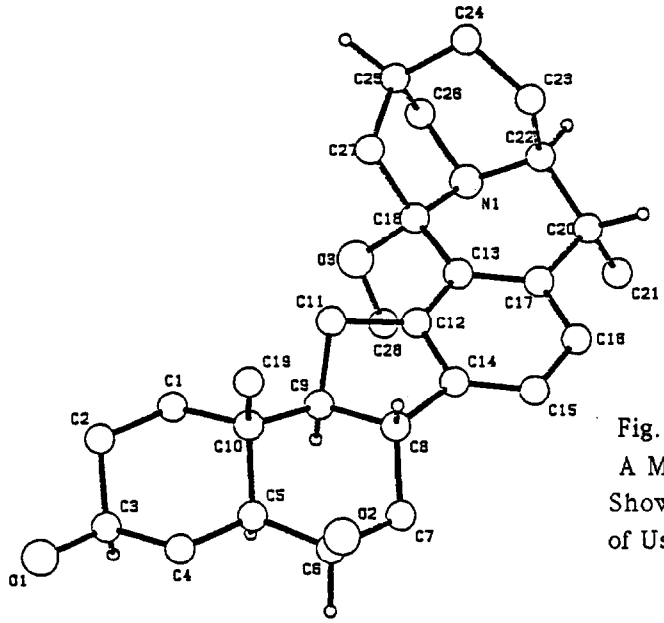


Fig. 3
A Molecular Perspective Drawing Showing the Absolute Configuration of Ussurienine

1 is the first example of the 5α-cevanine alkaloid with the aromatized D-ring, and an extra five membered ring formed between C-18 and C-27. The biogenesis, pharmacological activity, and the distribution of this alkaloid among Fritillaria species are now under investigation.

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