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ISOLATION AND STRUCTURE ELUCIDATION OF NEW ALKALOIDS FROM FRITILLARIA DELAVAYI FRANCH

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Two new D/E cis-5α-cevanine alkaloids, delavine(1) and delavinone(2), which lack a hydroxyl group at C-20, have been isolated together with imperialine from *Fritillaria delavayi* Franch (Liliaceae). The X-ray crystallographic analysis of 2 also has been accomplished.

KEYWORDS —— Liliaceae; bei mu; *Fritillaria delavayi*; 20-deoxy-5α-cevanine; D/E cis juncture; X-ray analysis

Of the present Chinese herbal drugs, those from the *Fritillaria* species (Bei mu) have been therapeutically used as antitussives, expectrants, and hypotensives.[1)] Two new alkaloids, delavine(1) and delavinone(2), have been isolated with imperialine(3), by silica gel column chromatography, from the crude aglycone fraction of the MeOH extract of *F. delavayi* Franch (Liliaceae),[2)] which is commercially available in markets in China.

Delavine(1) was crystallized from MeOH as colorless needles, mp 182-183 °C, $C_{27}H_{45}NO_2$ (m/z 415.3449, determined by high resolution mass spectrometry), $[\alpha]_D$ -20.0 ° (c 0.5, $CHCl_3$), IR: $\nu_{max}^{CHCl_3}$ cm^{-1} 3550-3250(OH), 2800 and 2750(trans-quinolizidine moiety),[3)] MS m/z: 415(M^+, 43%), 400(9%), 397(3%), 112(62%), 111(base peak). In the ^{1}H-NMR spectrum of 1 ($CDCl_3$), the tertial methyl group at δ 1.01(3H, s) is shifted downfield, compared with that of 5α-cholestanol, because of the 1,3-diaxial interaction with the β-axial hydroxyl group. Two signals at δ 3.65(1H, m, $w\frac{1}{2}$=23Hz), and 3.84(1H, br s, $w\frac{1}{2}$=8Hz) are the hydrogens on the carbon bearing hydroxyl group (on acetylations these signals shifted downfield at δ 4.70 and 4.97 respectively). This spectral behavior was similar to that of 19-H, 3α-H and 6α-H in isoverticine(4), which was isolated from *F. verticillata* var. *thumbergii*.[4)]
The chemical shift and coupling constant at δ 1.09(3H,d, J=7Hz) suggests the presence of a β-axial methyl group at C-25, namely the (25S)-configuration. On the other hand, the appearance of the signal at δ 0.83(3H, d, J=7Hz, 21-H) shows an absence of the hydroxyl group at C-20. These results suggest that 1 possesses a (25S)-5α-cevanine skeleton, with two substitutional hydroxyl groups at 3β and 6β, and none at C-20.

Table I.
^{13}C Chemical Shifts of Cevanine Alkaloids$^{a)}$

C-No.	1	2	3	4	5	6
1	39.4*^{1}	37.6	37.6	38.8	38.1	37.1
2	31.4	30.6*^{1}	30.6*^{1}	31.2	31.4*^{1}	30.5*^{1}
3	71.9	70.9	70.9	71.9	72.0	70.9
4	34.8	30.3*^{1}	30.4*^{1}	35.5	41.8	30.1*^{1}
5	48.1	56.7	56.4*^{2}	48.3	142.4	56.5*^{2}
6	73.2	211.0	210.7	72.6	122.3	211.0
7	39.6*^{1}	47.0	46.9	39.1	31.2*^{1}	46.0
8	36.7	39.7*^{2}	40.3	35.8	38.6	42.1
9	57.9	56.7	56.7*^{2}	57.5	54.4	56.7*^{2}
10	35.5	38.3	38.2	35.5	37.0	38.4
11	30.8*^{3}	30.0*^{1}	29.9*^{3}	29.6*^{1}	30.3*^{2}	29.4*^{3}
12	39.1*^{2}	39.5*^{2}	39.2	41.0	41.5	41.1
13	39.1*^{2}	39.3*^{2}	34.3	39.3	37.9	39.3
14	41.2*^{4}	41.0	42.1	43.8	45.3*^{3}	43.5
15	28.7	26.8	26.9	24.9	25.1*^{4}	24.7
16	17.7	17.1	18.8	20.9	24.9*^{4}	20.6
17	41.6*^{4}	46.9	46.6	49.0	45.5*^{3}	48.8
18	59.2	59.3	59.9	61.9*^{2}	62.6*^{5}	61.8*^{4}
19	15.7	12.7	12.7	15.0	19.1	12.8
20	38.9*^{2}	35.7	72.0	71.1	36.2	71.0
21	14.7	15.6	22.4	20.5	8.6	20.4
22	62.5	62.4	63.5	70.5	68.0	70.3
23	25.0	24.9	19.7	19.1	24.3*^{4}	19.1
24	30.3*^{3}	30.4*^{1}	29.6*^{3}	29.5*^{1}	28.9*^{2}	29.2*^{3}
25	28.4	28.4	27.8	27.8	28.3	27.7
26	61.7	61.8	61.4	62.6*^{2}	63.9*^{5}	62.3*^{4}
27	18.3	18.3	17.3	17.4	17.9	17.3

a) In ppm relative to TMS in $CDCl_3$. Assignment of chemical shifts marked with the same number of asterisks may be reversed.

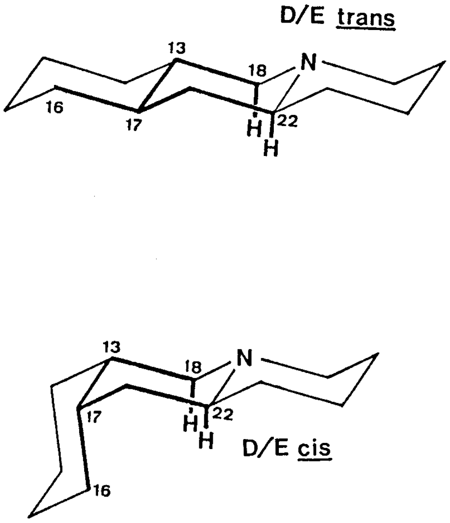
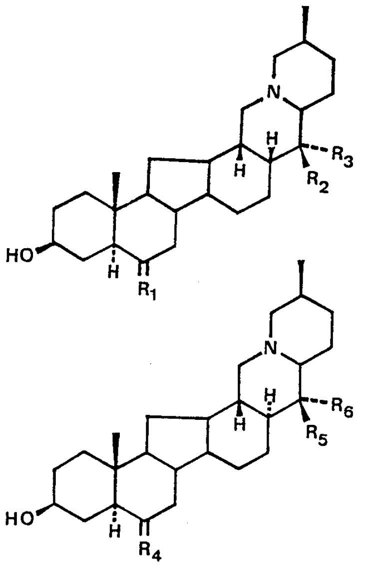
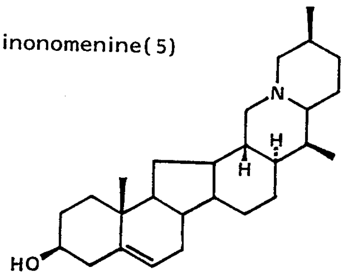


Fig.1



shinonomenine(5)



delavine (1) : $R_1={<}^{OH}_{H}$, R_2=H, R_3=CH_3

delavinone (2) : R_1=O, R_2=H, R_3=CH_3

imperialine (3) : R_1=O, R_2=OH, R_3=CH_3

isoverticine (4) : $R_4={<}^{OH}_{H}$, R_5=OH, R_6=CH_3

verticinone (6) : R_4=O, R_5=OH, R_6=CH_3

Fig.2

Yunusov et al. were the first to isolate 20-deoxy-5α-cevanine-3,6-diol type alkaloids from Fritillaria genus (edpetilidine and eduardinine).[5,6] However their structures were mainly determined only from the MS and ^{1}H-NMR spectra, and were not confirmed.

The assignment of the 25MHz ^{13}C-NMR spectrum of **1** is shown in Table I. The chemical shifts from C-1 to C-12 and C-19 in **1** correspond with those of isoverticine(**4**), while the shifts from C-23 to C-27 in **1** correspond with those of shinonomenine(**5**), which was isolated from Veratrum grandiflorum Loesen.[4] The chemical shifts of C-18, C-22, which are carbons attached to a nitrogen atom, and C-16 exhibited upfield shifts at δ 59.2, 62.5 and 17.7 compared with those of **5**, at δ 62.6, 68.0 and 24.9 respectively. These observations in **1** appear in the presence of the D/E cis juncture, because of the 1,3-diaxial interactions between the C-16-C-17 bond and the two hydrogens at C-22 and C-18(Fig. 1).[7] As the first example, imperialine (or Kashmirine), which has the D/E cis juncture, has already been isolated from a natural source.[8]

Delavinone(**2**) was crystallized from MeOH as colorless needls, $C_{27}H_{43}NO_2$ (M^+, m/z 413.3267), mp 182-184°, $[\alpha]_D$ -54.0° (c 0.5, $CHCl_3$), IR: $\nu^{CHCl_3}_{max}$ cm^{-1}, 3600 (OH), 2800 and 2750 (trans-quinolizidine moiety), 1700(six membered ring ketone), CD: $[\theta]_{295}$ -4700 (negative maximum), MS: m/z 413 (M^+,47%), 398(10%), 395(2%), 112(50%), 111(base peak), ^{1}H-NMR ($CDCl_3$) δ: 0.75(3H, s, 19-H), 0.83(3H, d, J=7Hz, 21-H), 1.09(3H, d, J=7Hz, 27-H), 3.56(1H, m, $w\frac{1}{2}$=23Hz, 3α-H). These spectral properties of **2** are similar to those of **1**, and so the fundamental structure of **2** appears to include (25S)-20-deoxy-5α-cevanin. On the other hand, the ^{1}H-NMR shift of 19-H in **2** exhibited an upfield shift to δ 0.75 compared with that in **1**, and similar to that in verticinone(**6**).[4] The location of a carbonyl group at C-6 was confirmed by the CD spectrum, which indicates a negative maximum at 295 nm for 6-keto steroids.[9] Delavinone(**2**) was converted to **1** by reduction with $NaBH_4$.

The molecular structure of **2** was confirmed by an X-ray crystal analysis of its hydrochloride (mp 217-219°C). The structure of the molecule is shown in Fig. 3.

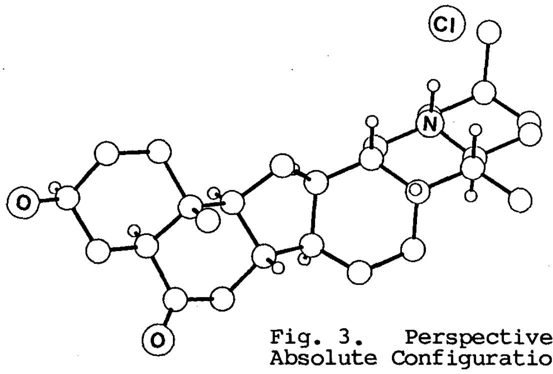


Fig. 3. Perspective Drawing of the Molecule Showing the Absolute Configuration of Delavinone-Hydrochloride

The crystal of delavinone-hydrochloride belongs to the monoclinic system with space group $P2_1$, and the cell dimensions, a=15.223(2), b=7.278(1), c=11.555(3)Å, β=91.68°(1), z=2. Three-dimensional intensity data were collected on Rigaku AFC-5 diffractometer, and 2002 independent refractions were measured for $\theta \leq 60°$ by the θ-2θ scan technique with Cu-Kα radiation. The structure was solved by a conventional heavy-atom method and refined by a block-diagonal anisotropic least-squares technique to R=0.040 for 1557 reflections. The absolute configuration was determined based on the β-configulation of the C-19. All six-membered rings are in the chair conformation except the D ring which assumes a twisted chair form. The ring junctures are as follows: A/B *trans*, B/C *trans*, C/D *cis*, D/E *cis*, and E/F *trans*. The configurations at other chiral centers have been determined to be 3-OH equatorial, 10-Me axial, 20-Me equatorial, 25-Me axial and a lone pair on nitrogen axial. All these groups are in the β-orientation, except 20-Me which is in the α.

These two *Fritillaria* alkaloids **1** and **2** are the first examples of D/E *cis*-20-deoxy-5α-cevanine alkaloids, and their absolute configurations were established. The pharmacological activities of *F. delavayi* and its component alkaloids **1**, **2** and **3** (yields: 20, 10 and 2 ppm), and the distribution of these compounds among the *Fritillaria* genus, are now under investigation.

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