

## Communications to the Editor

[Chem. Pharm. Bull.]  
33(6)2614-2617(1985)

ISOLATION AND STRUCTURE ELUCIDATION OF NEW ALKALOIDS  
FROM Fritillaria delavayi FRANCH

Ko Kaneko,<sup>\*,a</sup> Takao Katsuhara,<sup>a</sup> Hiroshi Mitsuhashi,<sup>a</sup> Yuh-Pan Chen,<sup>b</sup>  
Hong-Yen Hsu<sup>b</sup> and Motoo Shiro<sup>c</sup>

Faculty of Pharmaceutical Sciences, Hokkaido University,<sup>a</sup> Kita-12-jo Nishi-6-  
chome Kita-ku, Sapporo 060, Hokkaido, Japan, Brion Research Institute of  
Taiwan,<sup>b</sup> Taipei, Taiwan 107, Republic of China, and Shionogi Research  
Laboratory, Shionogi & Co.,<sup>c</sup> Fukushima-ku Osaka 553, Japan

Two new D/E cis-5 $\alpha$ -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-5 $\alpha$ -  
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.<sup>1)</sup> 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),<sup>2)</sup> which is commercially available in markets in China.

Delavine(1) was crystallized from MeOH as colorless needles, mp 182-183°C, C<sub>27</sub>H<sub>45</sub>NO<sub>2</sub>  
( $m/z$  415.3449, determined by high resolution mass spectrometry), [ $\alpha$ ]<sub>D</sub> -20.0° ( $c$  0.5,  
CHCl<sub>3</sub>), IR:  $\nu_{\text{max}}^{\text{CHCl}_3}$  3550-3250(OH), 2800 and 2750(trans-quinolizidine moiety),<sup>3)</sup> MS  
 $m/z$ : 415(M<sup>+</sup>, 43%), 400(9%), 397(3%), 112(62%), 111(base peak). In the <sup>1</sup>H-NMR spectrum of  
1 (CDCl<sub>3</sub>), the tertial methyl group at  $\delta$  1.01(3H, s) is shifted downfield, compared with  
that of 5 $\alpha$ -cholestanol, because of the 1,3-diaxial interaction with the  $\beta$ -axial hydroxyl  
group. Two signals at  $\delta$  3.65(1H, m,  $w_{1/2}$ =23Hz), and 3.84(1H, br s,  $w_{1/2}$ =8Hz) are the hydrogens  
on the carbon bearing hydroxyl group (on acetylations these signals shifted downfield at  $\delta$   
4.70 and 4.97 respectively). This spectral behavior was similar to that of 19-H, 3 $\alpha$ -H and  
6 $\alpha$ -H in isovorticine(4), which was isolated from F. verticillata var. thumbergii.<sup>4)</sup>  
The chemical shift and coupling constant at  $\delta$  1.09(3H, d, J=7Hz) suggests the presence of a  
 $\beta$ -axial methyl group at C-25, namely the (25S)-configuration. On the other hand, the  
appearance of the signal at  $\delta$  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)-5 $\alpha$ -cevanine skeleton, with  
two substitutional hydroxyl groups at 3 $\beta$  and 6 $\beta$ , and none at C-20.

Table I.  
 $^{13}\text{C}$  Chemical Shifts of Cevanine Alkaloids<sup>a)</sup>

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

a) In ppm relative to TMS in  $\text{CDCl}_3$ . Assignment of chemical shifts marked with the same number of asterisks may be reversed.

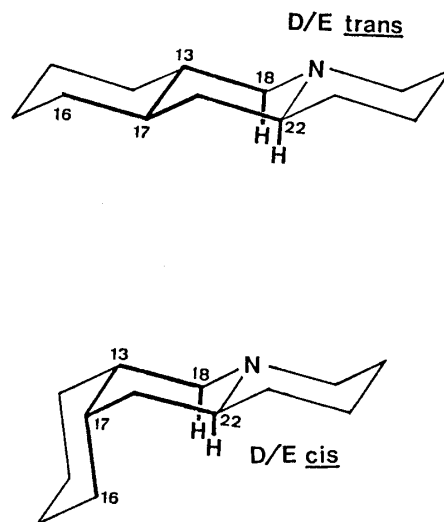


Fig.1

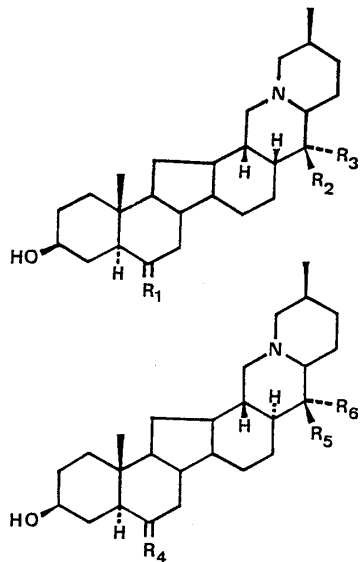
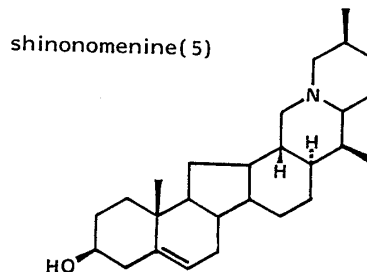


Fig.2



shinonomenine(5)

- delavine (1) :  $\text{R}_1 = \begin{array}{c} \text{OH} \\ \diagup \\ \text{H} \end{array}$ ,  $\text{R}_2 = \text{H}$ ,  $\text{R}_3 = \text{CH}_3$   
 delavinone (2) :  $\text{R}_1 = \text{O}$ ,  $\text{R}_2 = \text{H}$ ,  $\text{R}_3 = \text{CH}_3$   
 imperialine (3) :  $\text{R}_1 = \text{O}$ ,  $\text{R}_2 = \text{OH}$ ,  $\text{R}_3 = \text{CH}_3$   
 isovorticine (4) :  $\text{R}_4 = \begin{array}{c} \text{OH} \\ \diagup \\ \text{H} \end{array}$ ,  $\text{R}_5 = \text{OH}$ ,  $\text{R}_6 = \text{CH}_3$   
 verticinone (6) :  $\text{R}_4 = \text{O}$ ,  $\text{R}_5 = \text{OH}$ ,  $\text{R}_6 = \text{CH}_3$

Yunusov *et al.* were the first to isolate 20-deoxy-5 $\alpha$ -cevanine-3,6-diol type alkaloids from *Fritillaria* genus (edpetilidine and eduardinine).<sup>5,6)</sup> However their structures were mainly determined only from the MS and <sup>1</sup>H-NMR spectra, and were not confirmed.

The assignment of the 25MHz <sup>13</sup>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 isovorticine(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.<sup>4)</sup> The chemical shifts of C-18, C-22, which are carbons attached to a nitrogen atom, and C-16 exhibited upfield shifts at  $\delta$  59.2, 62.5 and 17.7 compared with those of 5, at  $\delta$  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).<sup>7)</sup> As the first example, imperialine (or Kashmirine), which has the D/E *cis* juncture, has already been isolated from a natural source.<sup>8)</sup>

Delavinone(2) was crystallized from MeOH as colorless needles, C<sub>27</sub>H<sub>43</sub>NO<sub>2</sub> (M<sup>+</sup>,  $m/z$  413.3267), mp 182-184°, [ $\alpha$ ]<sub>D</sub> -54.0° ( $c$  0.5, CHCl<sub>3</sub>), IR:  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup>, 3600 (OH), 2800 and 2750 (*trans*-quinolizidine moiety), 1700(six membered ring ketone), CD: [ $\theta$ ]<sub>295</sub> -4700 (negative maximum), MS:  $m/z$  413 (M<sup>+</sup>, 47%), 398(10%), 395(2%), 112(50%), 111(base peak), <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 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*,  $w_{1/2}$ =23Hz, 3 $\alpha$ -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-5 $\alpha$ -cevanin. On the other hand, the <sup>1</sup>H-NMR shift of 19-H in 2 exhibited an upfield shift to  $\delta$  0.75 compared with that in 1, and similar to that in verticinone(6).<sup>4)</sup> 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.<sup>9)</sup> Delavinone(2) was converted to 1 by reduction with NaBH<sub>4</sub>.

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.

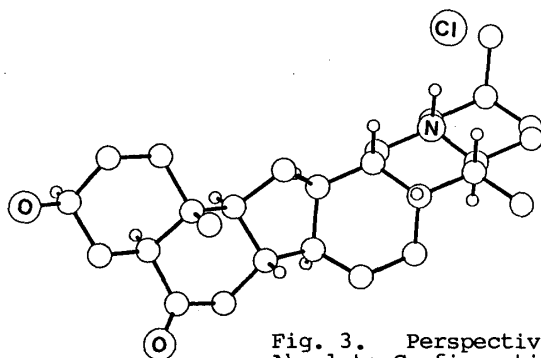


Fig. 3. Perspective Drawing of the Molecule Showing the Absolute Configuration of Delavinone-Hydrochloride

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,  $\beta$ =91.68°(1),  $z$ =2. Three-dimensional intensity data were collected on Rigaku AFC-5 diffractometer, and 2002 independent reflections were measured for  $\theta \leq 60^\circ$  by the  $\theta$ -2 $\theta$  scan technique with Cu-K $\alpha$  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  $\beta$ -configuration 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  $\beta$ -orientation, except 20-Me which is in the  $\alpha$ .

These two Fritillaria alkaloids 1 and 2 are the first examples of D/E cis-20-deoxy-5 $\alpha$ -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.

ACKNOWLEDGEMENT      The Authors wish to thank Professor Sho Ito (Tohoku Univ., Sendai) for identification of imperialine.

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(Received April 25, 1985)