STEROID ALKALOIDS OF FRESH BULBS OF FRITILLARIA THUNBERGII MIQ. AND OF CRUDE DRUG "BAI-MO" PREPARED THEREFROM

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Abstract ----- A new minor alkaloid FR-5 was isolated, besides verticine (I) and verticinone (II), from the fresh bulbs of Fritillaria thunbergii Miq., and the structure was determined as III. The crude drug "Bai-mo", which was prepared from the above bulbs by treatment with lime followed by bleaching in the sun, provided two alkaloids F-3 and -4, together with I and II, in the free state, and additional two F-5 and -6 in the form of their acetates. They were assigned the structures VIII, IX, X and XI, which correspond, respectively, to an oxidation product of I, II and III.

A crude drug "Fritillariae Bulbs" ("Beimu" or "Pei-mu" in Chinese and "Bai-mo" in Japanese) has long been known as a principal Chinese medicine. Among various kinds of "Beimu", processed bulbs of more than fifteen species of Fritillaria plants (Liliaceae),[1] "Zhebeimu" and Japanese "Bai-mo" are prepared from the bulbs of F. thunbergii Miq. (F. verticillata Willd. var. thunbergii (Miq.) Baker).

As for the constituent alkaloids of the fresh bulbs of F. thunbergii grown in Japan, Fukuda[2] isolated four compounds, verticine (I), fritillarine, verticilline and an amorphous base, and Morimoto and Kimata[3] obtained peimine and its glucoside, peiminoside. Later, I was identified with peimine, and the identity of fritillarine and peiminine[4] with verticinone (II) derived from I was also evidenced.[5] The structures of I and II were determined by Itô, et al.[5]

The present study was conducted, for one thing, in the hope of isolation of new alkaloids from the fresh bulbs of F. thunbergii and, for another, to examine the basic constituents of "Bai-mo", which have not so far been reported, but which are of interest in that they might possibly be the compounds somewhat modified in structures from those of fresh bulbs due to processing.

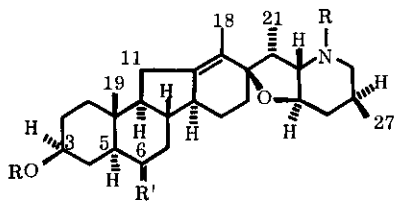
Dedicated to Professor Dr. T. Kametani on the occasion of his retirement from Tohoku University.

The fresh bulbs (19.6 kg), cultivated in Nara prefecture and collected in May, have provided a new alkaloid FR-5 (42 mg) along with I (740 mg) and II (410 mg).[6]

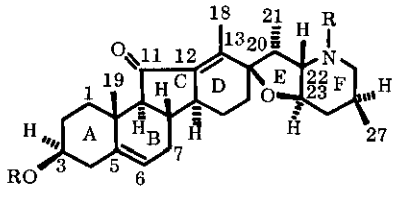
FR-5 (III), colorless needles (from MeOH), mp 255°, $[\alpha]_D$ -33.2° (c=1.0, $CHCl_3$)[7], $C_{27}H_{41}O_3N$, showed in the EI-mass (MS) spectrum the molecular ion at m/z 427 and the fragment peaks at 412, 314, 125, 124, and 110 (base peak). The spectrum was similar to that of jervine (IV)[8,9] (MS m/z: 425 ($M^{+\cdot}$), 410, 396, 314, 125, 124, 110 (base peak, due to F-ring[10])) indicating a resemblance of III to IV in structure. The IR spectrum of III showed an absorption of six-membered ring ketone (1695 cm^{-1}) instead of those of 12-en-11-one (1705 and 1630) in IV. III was acetylated to give diacetate (V), colorless fine needles, mp 231-232° (from AcOEt-hexane), $[\alpha]_D$ +7.2° (c=1.0, $CHCl_3$). IR (KBr, cm^{-1}): 1730 (OAc), 1700 (C=O), 1660 (NAc). MS m/z: 511 ($M^{+\cdot}$) ($C_{31}H_{45}O_5N$), 496, 468, 167 (base peak), 152. The above results and the ^{1}H-NMR (100 MHz) (PMR) (in $CDCl_3$) spectral data of III and V in comparison with those of IV and its acetate (VI) (Table I) suggested that III is an 11-deoxo-5,6-dihydrojervine having one carbonyl function at C_1, C_7 or most probably at C_6. When III was reduced with $NaBH_4$, a diol (VII), mp 198°, was yielded, and the PMR spectrum, in comparison with that of III, showed a downfield shift of 19-CH_3 signal by 0.28 ppm, implying that the hydroxyl group provided has 1,3-diaxial relationship with 19-CH_3 group. Thus, two hydroxyl groups in VII are located at C_6 (β, axial) and C_3. The signals of 19-CH_3 group and 3-H in the PMR spectra of III (Table I) and

Table I. PMR Spectral Data (δ, ppm, $CDCl_3$) of III - VI (cf. ref. 9, 11)

	18-CH_3	19-CH_3	21-CH_3	27-CH_3	3-H	6-H
FR-5 (III)	1.66 (s)	0.69 (s)	0.94 (d, J=7 Hz)	0.94 (d, J=7 Hz)	3.54 (br.m, $W_{1/2}$ =28 Hz)	
jervine (IV)	1.79 (s)	1.01 (s)	0.94 (d, J=7 Hz)	0.94 (d, J=7 Hz)	3.54 (br.m, $W_{1/2}$ =24 Hz)	5.36 (m)
III acetate (V)	1.72 (s)	0.71 (s)	1.05 (d, J=7 Hz)	0.87 (d, J=7 Hz)	4.68 (br.m, $W_{1/2}$ =24 Hz)	
IV acetate (VI)	2.19 (s)	1.03 (s)	1.05 (d, J=7 Hz)	0.87 (d, J=7 Hz)	4.67 (br.m, $W_{1/2}$ =24 Hz)	5.40 (m)



FR-5 (III) : R=H, R' = =O
V : R=Ac, R' = =O
VII : R=H, R' = 



jervine (IV) : R=H
VI : R=Ac

VII (19-CH_3, 0.97; 3-H, 3.64 ppm, br.m, in $CDCl_3$) were in accordance with those of 3β-hydroxy-5α-cevanine derivatives II (cf. Table II) and isoverticine[6], respectively.

Consequently III is defined as 11-deoxo-6-oxo-5α,6-dihydrojervine.

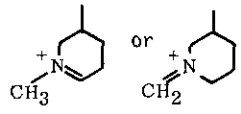
The crude drug "Bai-mo" (20 kg) (product of Nara prefecture), prepared from the afore-mentioned bulbs by treatment with lime followed by bleaching in the sun, afforded, besides I (1.86 g) and II (0.93 g), two new compounds F-3 (VIII) (3.05 g), colorless needles (from AcOEt-hexane), mp 283-288° (dec.), $[\alpha]_D$ +8.4° (c=1.9, MeOH), $C_{27}H_{45}O_4N$ (MS m/z: 447 ($M^{+\cdot}$)), and F-4 (IX) (1.18 g), colorless prisms (from AcOEt-hexane), mp 283-285° (dec.), $[\alpha]_D$ -54.5° (c=1.8, MeOH), $C_{27}H_{43}O_4N$ (MS m/z: 445 ($M^{+\cdot}$)), together with two compounds F-5 and -6 in the form of their acetates.

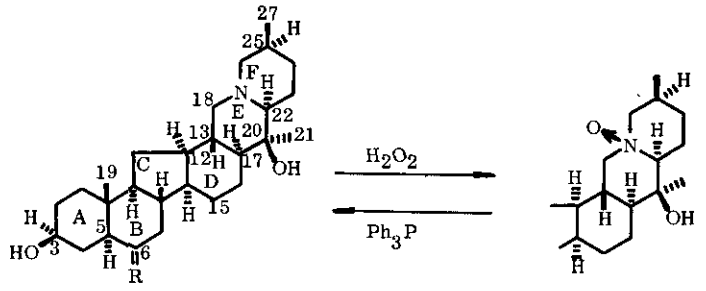
The mass and PMR spectral data of VIII and IX in comparison with those of I and II (Table II) suggested that VIII and IX are respectively an analog of I and II bearing one more oxygen atom in F-ring. Moreover, the IR spectra (in CH_2Cl_2) of I and II showed the absorption bands at 2800 - 2700 cm^{-1} characteristic to trans quinolizidine moiety,[13] while in those of VIII and IX they were lacking, therefore VIII and IX were supposed to be N-oxides of I and II. The presumption was confirmed by the facts that VIII and IX were reduced with Ph_3P in AcOH[14] to yield I and II, and that I and II were oxidized with H_2O_2 and NaOH in MeOH to give VIII and IX, respectively.[15] Comparing with the PMR spectral data of I and II (Table II), the 27-CH_3 signals of VIII and IX are shifted downfield by 0.29 ppm, but the 21-CH_3 signals shifted only slightly (0.05 - 0.03 ppm). The above results are properly interpreted only by assignment of β-axial configuration at the N asymmetric center and trans E/F ring juncture to VIII and IX (Fig. 1).[16]

Table II. Mass and PMR Spectral Data of VIII, IX, I and II

	mass fragment (m/z) (cf. ref. 10)	PMR (δ, ppm, CD_3OD) (cf. ref. 6, 12)				
		19-CH_3	21-CH_3	27-CH_3	3-H	6-H
F-3 (VIII)	447 ($M^{+\cdot}$), 431, 430, 412 386, 114, 112*, 110	0.85 (s)	1.10 (s)	1.42 (d, J=7 Hz)	3.45 (br.m)	3.45 (br.m)
verticine (I)	431 ($M^{+\cdot}$), 413, 386 112*, 98	0.84 (s)	1.05 (s)	1.13 (d, J=7 Hz)	3.45 (br.m)	3.45 (br.m)
F-4 (IX)	445 ($M^{+\cdot}$), 429, 428, 410 384, 114, 112*, 110	0.78 (s)	1.09 (s)	1.42 (d, J=7 Hz)	3.45 (br.m)	
verticinone (II)	429 ($M^{+\cdot}$), 411, 384 112*, 98	0.78 (s)	1.06 (s)	1.13 (d, J=7 Hz)	3.52 (br.m)	

* base peak (cf. ref. 10)





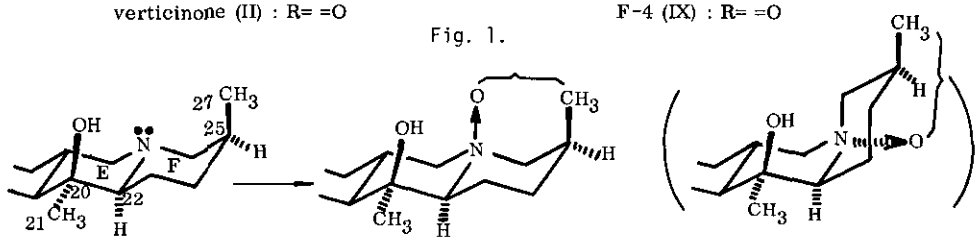
verticine (I) : R= $\overset{OH}{H}$

verticinone (II) : R= =O

F-3 (VIII) : R= $\overset{OH}{H}$

F-4 (IX) : R= =O

Fig. 1.



F-5 acetate (X), colorless needles (23 mg, from acetone-hexane), mp 214-216°, $[\alpha]_D$ +21.7° (c=1.0, $CHCl_3$), exhibited in its mass spectrum the peaks at m/z 527 ($M^{+\cdot}$), 512, 484, 291, 222, 167 (base peak) and 152, the former three of which are 16 mass units more than the corresponding peaks observed in the spectrum of V, while the latter two of which are same as those of V, suggesting that X has additional one oxygen atom in A - E rings of III. X showed no IR absorption of hydroxyl group and the PMR spectra of X and V were in good agreement except for the δ values of 18-CH_3 signals (1.30 ppm in X and 1.72 in V).

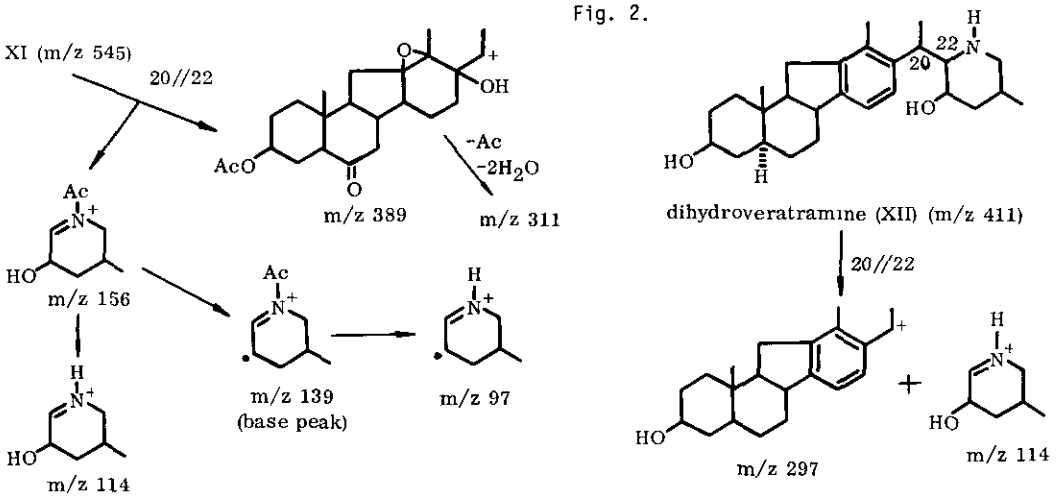
Thus, X is assigned the structure 12,13-epoxy-11-deoxo-6-oxo-5α,6-dihydrojervine N,O-diacetate.

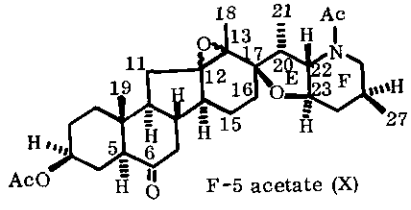
F-6 acetate (XI), a white powder (36 mg, from hexane), mp 161-163°, $[\alpha]_D$ +20.1° (c=0.8, $CHCl_3$), exhibited in its PMR spectrum two methyl singlets, two methyl doublets, N- and O-acetyl signals having the same δ values as those of X and a broad multiplet (br.m) ($W_{1/2}$ =8 Hz) ascribable to a proton geminal to hydroxyl group. The IR spectrum was different from that of X in showing the absorption of hydroxyl group in addition to those of N- and O-acetyl and six-membered ring ketone. In the mass spectrum of XI the molecular ion at m/z 545 (18 mass units more than that of V) and the fragment peaks at 389, 311, 156, 139 (base peak), 114 and 97 were observed. Based on these data and taking co-existence of X into account, XI was thought to be a compound which has the E-ring of X opened, and the mass fragments mentioned above were assumed, in analogy to the mode of cleavage of dihydroveratramine (XII)[10], to have the structures shown in Fig. 2.

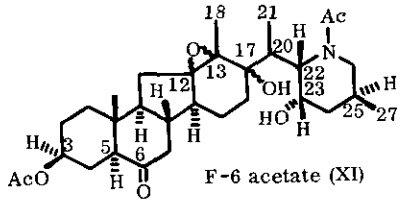
The splitting pattern of 23-H signal in the PMR spectrum indicated the α-axial configuration of 23-OH[17], being epimeric to that of veratramine.[9]

Now XI is regarded to be 12,13-*epoxy*-22S,25S,5α-*veratranine*-3β,17,23α-triol-6-one N,O(3)-diacetate. Since the fraction containing compounds F-5 and -6 showed neither N- nor O-acetyl absorptions in the IR spectrum, they are considered to exist originally as the free hydroxyalkamines corresponding to X and XI, respectively.

Fig. 2.







Compounds F-3 (VIII) and -4 (IX) were much less toxic and more hypotensive than I and II in mice. It is worthy of note that the alkaloids so far isolated from the fresh bulbs are considerably or completely oxidized during treatment with lime followed by bleaching in the sun, and that the processing seems to be of pharmacological significance. Compound FR-5 (III) is, to our knowledge, a new natural 5α-jervanine alkaloid.

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References and Footnotes

1. Pharmaceutical Institute, Chinese Academy of Medical Science, and others (ed.), "Zhong Yao Zhi", Vol. 1, Peking, 1979, p. 93.
2. M. Fukuda, Nippon Kagaku Zasshi, 1929, 50, 74; Sci. Rept. Tohoku Univ., Ser. A, 1929, 18, 323 ; Nippon Kagaku Zasshi, 1948, 69, 165.
3. H. Morimoto and S. Kimata, Chem. Pharm. Bull., 1960, 8, 302, 871.
4. T.-Q. Chou and K.-K. Chen, Chinese J. Physiol., 1932, 6, 265.
5. a) S. Itô, M. Kato, K. Shibata, and T. Nozoe, Chem. Pharm. Bull., 1961, 9, 253; ibid., 1963, 11, 1337; b) S. Itô, Y. Fukazawa, T. Okuda, and Y. Iitaka, Tetrahedron Lett., 1968, 5373.
6. Isolation and characterization of isoverticine (6-epimer of I) have recently been reported (K. Kaneko, M. Tanaka, K. Haruki, H. Naruse, and H. Mitsuhashi, Tetrahedron Lett., 1979, 3737; K. Kaneko, N. Naruse, K. Haruki, and H. Mitsuhashi, Chem. Pharm. Bull., 1980, 28, 1345.
7. Optical rotations were taken at 18 - 20° by using 1 dm cell.
8. Isolated in this laboratory (T. Tsukamoto and A. Yagi, Yakugaku Zasshi, 1959, 79, 1102).
9. J. Tomko and Z. Votický, "The Alkaloids", Vol. 14, ed. by R. H. F. Manske, Academic Press, Inc., New York, London, 1973, p. 5.
10. H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 2, Holden-Day, Inc., San Francisco, London, Amsterdam, 1964, p.13.
11. K. Nakanishi, T. Goto, S. Itô, S. Natori, and S. Nozoe (ed.), "Natural Products Chemistry", Vol. 2, Kodansha Ltd., Tokyo, and Academic Press, Inc., New York, London, 1975, p. 273.
12. a) S. Itô, J. B. Stothers, and S. M. Kupchan, Tetrahedron, 1964, 20, 913; b) S. Itô, Y. Fukazawa, and M. Miyashita, Tetrahedron Lett., 1976, 3161.
13. F. Bohlmann, Angew. Chem., 1957, 69, 641; Chem. Ber., 1958, 91, 2157.
14. L. Horner and H. Hoffmann, Angew. Chem., 1956, 68, 480.
15. An N-oxide of imperialine (17-epimer of II)[12b] was isolated from aerial parts of a Fritillaria plant and it was not identical to the synthetic one provided by oxidation of imperialine with H_2O_2 (D. M. Harrison, "The Alkaloids", Vol. 8, ed. by M. F. Grundon, The Chemical Society, London, 1978, p. 262.
16. The molecular structure of II methobromide, chair conformations of all six-membered rings, ring junctures of A/B(tr), B/C(tr), C/D(cis), D/E(tr) and E/F(cis), and the configurational change from E/F trans in II to cis on quarternization were evidenced by X-ray analysis.[5b]
17. It is assumedly due to any hindrance by, for instance, 21-CH_3 group that 23-OH remains unacetylated.

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