On the Alkaloid of *Fritillaria verticillata* WILD. var. *Thunbergii*  
Baker. II.1) The Structure of Verticine.2)

We wish to provide herein the evidence which permits the assignment of the structure (I) for verticine,3)1 only the main alkaloid of *Fritillaria verticillata* var. *Thunbergii,*3 together with some modification of the partial structure proposed previously.

We have shown the presence of two secondary and a tertiary hydroxyls in I,4) diacetate (II), C_{23}H_{44}O_{8}N, 5) m.p. 117–120°, by successive oxidation of I to verticinone (III)6), acetate (IV), C_{29}H_{46}O_{8}N, m.p. 173–174°, and to verticindione (V).7) Although V, and also III, were converted to I by sodium in ethanol or lithium in liquid ammonia in the presence of methanol, the reduction of both ketones with sodium borohydride, lithium aluminum hydride or aluminum isopropoxide as well as by catalytic reduction (Pt, ethanol) resulted in the formation of isoverticine (VI), C_{27}H_{40}O_{6}N, m.p. 206–214°, pKa’ 9.4; diacetate (VII), C_{29}H_{48}O_{8}N, m.p. 124°. Since both I and VI afforded V on chromic acid oxidation, they are epimeric at one of the secondary hydroxyls. It was also assumed from the mode of formation in these reductions8,9) that the two hydroxyls are both equatorial in I, while they are equatorial and axial in VI. This assumption was supported by the following observations: i) VI had a larger Rf values than I in paper and thin layer chromatography.5,6) ii) VI, contrary to I, was easily oxidized by two moles of N-bromosuccinimide to develope a carbonyl band at 1705 cm^{-1}.7) iii) I was

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1) Presented at the 6th Symposium on Natural Product held at Sapporo on July 6th, 1962.
2) The identity of I with peimine isolated by Chou and Chen1) was established by the direct comparison carried out by Dr. J.H. Chu, Institute of Organic Chemistry, Academia Sinica, Shanghai, thus the presence of ceyane skeleton being established in I. We are deeply indebted to Dr. Chu for his kind determination of the mixed melting point. We use the name “verteicine” in favor of “peimine” in this paper, since the former has first been named by Fukuda2) to the pure alkaloid, m.p. 223–224°. Elemental analyses of Fukuda’s original sample agreed with the formula C_{27}H_{40}O_{6}N, in our hand.
3) For the historical aspect of the study on the constituents of *Fritillaria* species, see H. Morimoto, S. Kimata : This Bulletin, 8, 302, 871 (1960), which also describe the isolation of peimine and its glucoside as well as some reaction of these compounds.
4) UV : λ_{max}^{(HCl)} 10. The value was erroneously printed in the reference 1.
5) All analytical values through this paper are in good agreement with the formulas shown.
the predominant component in the equilibrated mixture (sodium butoxide in butanol) both from I and VI.\textsuperscript{3,9}

The hydroxyl, which is thermodynamically more stable and more resistant to chromic acid, has been shown\textsuperscript{3} to be located at C-3 of the cevane ring system, since, among other indications, dehydrodeoxverticinone (VIII), derived from III via deoxverticinone (IX), has a positive Cotton effect. The $\beta$-configuration of this hydroxyl was also supported by the sharp and simple band at 1242 cm$^{-1}$ in the infrared spectrum of deoxverticinone acetate, C$_{23}$H$_{37}$O$_{4}$N, m.p. 183\textdegree C.\textsuperscript{9}

The other secondary hydroxyl is located at C-6 because i) both V and cholestan-3,6-dione underwent aerial oxidation in alkaline medium to develop an ultraviolet maximum ($\nu_{\text{max}}^{\text{max}}$ = 375 mp) due to the presence of a 4-ene-3,6-dione system in the product, ii) on heating with polyphosphoric acid, III yielded a cisoid $\alpha$,$\beta$-unsaturated ketone (XI), C$_{27}$H$_{41}$O$_{3}$N, m.p. 122~123\textdegree C, UV: $\lambda_{\text{max}}^{\text{E98}}$ 241 mp ($\varepsilon$ 6000), IR $\nu_{\text{max}}^{\text{max}}$ cm$^{-1}$: 1669,

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Partial structures & R & Verticine series\textsuperscript{a)} & 5α-Steroid\textsuperscript{11)} & 5α-Steroid & 5α-Steroid \textsuperscript{h)} \\
\hline
 & No. & 27-Me & 21-Me & 19-Me (A) & 19-Me (B) & $\delta_{5α}^{5α}$ \\
\hline
 & X & 8.94 (8) & 9.01 & 9.20 & 9.22\textsuperscript{b)} & -0.02 \\
\hline
 & IX & 8.92 (7) & 8.97 & 9.20 & 9.20 & 0.00 \\
\hline
 & VIII & 8.93 (6) & 9.00 & 9.00 & 8.98\textsuperscript{b)} & +0.02 \\
\hline
 & [H] I & 8.93 (7) & 8.98 & 9.19 & & -0.02\textsuperscript{c)} \\
\hline
 & [Ac] II & 8.93 (6) & 8.96 & 9.09 & & -0.04\textsuperscript{d)} \\
\hline
 & [H] VI & 8.91 (7) & 8.96 & 8.96 & 8.98 & -0.02 \\
\hline
 & [Ac] VII & 8.94 (7) & 8.99 & 8.99 & 8.99 & +0.00 \\
\hline
 & [H] III & 8.94 (6) & 8.99 & 9.24 & 9.21\textsuperscript{e)} & +0.03 \\
\hline
 & [Ac] IV & 8.97 (7) & 9.01 & 9.24 & 9.22 & +0.02 \\
\hline
 & V & 8.96 (6) & 9.01 & 9.07 & 9.05\textsuperscript{f)} & +0.02 \\
\hline
\end{tabular}
\end{table}

Mean values
\[8.94 \pm 0.03 \ (6.5 \sim 8) \ \text{Me/sec.} \ \text{in CDCl}_3 \text{ and expressed in } \tau \text{ values. Figures in parentheses denote coupling constant in cm/s.} \]

\text{b) Mean values.}
\text{c) Derived by subtracting the value (9.20) for 5α-pregnane-3β-ol\textsuperscript{11)} and the effect (+0.01)\textsuperscript{22)} of 6α-hydroxy from the value for verticine (8.19).}
\text{d) Derived by subtracting the mean value (9.17) for 5α-steroidal 3β-acetate\textsuperscript{11)} and the effect (0.04)\textsuperscript{22)} of 6α-acetoxy group from the value for verticine diacetate (9.09).}

1613(vs), and iii) heating with one mole of hydrazine hydrate converted V to a dihydro-
pyridazine derivative, C₇H₄ON₃, m.p. >300°, IR νmax cm⁻¹ : 3448, 1631.¹⁰

Nuclear magnetic resonance spectral analysis confirmed the conclusion derived from
the reactions described above. Chemical shifts for all the methyls in verticine and its
derivatives are listed in Table I. For comparison, the Table also shows the 19-methyl
signals in the 5α-steroid series¹¹) and the differences between the corresponding chemical
shifts in the two series.

As shown in the Table, each of the verticine derivatives exhibited three methyl
signals consisting of one doublet and two singlets. The doublet and one of the singlets
remain constantly in the range of 8.94±0.03 τ and 8.99±0.03 τ respectively, showing that
these methyls are remote from any of the secondary hydroxyls. The position of the
other singlet, on the other hand, is in the wider range (8.96~9.24 τ), depending on the
nature of the substituents at C-3 and C-6, and the following shifts are observed; the
down-field shift due to 3-ketone in VIII compared with the corresponding deoxy-compound,
bisdeoxoverticindione (X), C₇H₄ON, m.p. 121~122°, IR νmax cm⁻¹ : 3500, 1125, obtained
by the Huang Minion reduction of V; and that due to the 1,3-diaxial disposition of two
groups¹¹,¹²) in VI compared with the epimeric (I); and the up-field shift due to 6-ketone
in III and IV compared with IX.¹³) These characteristic changes in the chemical shifts,
as well as the coincidence of all of the methyl signals of the corresponding members
in both series, established the partial stereochemistry of I to be 5α-cevane-3β,6α-diol.

The tertiary hydroxyl group, shown by the bands near 3500 and 1125 cm⁻¹ in infra-
red spectra of II, IV, V, VII, VIII, and X, must be at either C-20 or C-25 because only
one methyl signal appears as a doublet in the nuclear magnetic resonance spectra of all
compounds examined (Table I). Of the two possible sites, C-25 was ruled out because
i) I, V, and VI had comparable basisity with cevine (pKα 9.48¹³), and ii) mass spectra
of I, V, VI, and VIII always displayed as a base peak the ion, 112 m/e, attributed to III,
besides their respective molecular ions. That the configuration of the tertiary hydroxyl
is axial, as in cevine,¹⁴) was implied by the basisity and deduced from the infrared
spectrum of X (in CCl₄) which showed only an intramolecularly hydrogen-bonded OH
band at 3540 cm⁻¹ (concentration-independent in 0.1~0.01 M).¹⁴)

The stereochemistry of C-27 methyl was concluded to be axial from the chemical
shifts and coupling constants of the doublets (Table I) which were quantitatively in
accordance with those of cevine derivatives.¹⁴)

The evidence given so far confirms structure (I) for verticine but with the stereo-
chemistry at positions C-8, C-9, C-12, C-14, C-16, and C-17 undetermined; however by
analogy with the steroid series and from biogenetic considerations it is suggested that
the stereochemistry at these positions is as depicted in I.

The optical rotatory dispersion curve of III,¹³) which is strikingly similar to that of
7-oxycholesteryl acetate¹⁷) and therefore is responsible for the erroneous proposal in
our previous paper,¹⁵) was not essentially changed by the addition of acid, showing a large
hindrance to ketal formation. The difference between the shape of the curves of III
and 6-oxycholesteryl acetate¹⁵) may be attributed to a change in conformation of ring
B due to the size of the ring C in these compounds.

12) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, K. Tsuda : This Bulletin, 10, 338
(1962).
Our deep gratitude is expressed to Mr. M. Kodama, Tohoku University, for his cooperation in some experiments, to Prof. J. B. Stothers, University of Western Ontario, for NMR measurement, and to Prof. C. Djerassi, Stanford University, for mass spectra.

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Received June 25, 1963  
Revised July 23, 1963

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UDC 547.918 : 615.711.5

Hydroxylation of Digitoxigenin Derivatives by Absidia orchidis

In the previous paper we reported the hydroxylation of digitoxigenin (I) at 1β, 5β, and 7β positions by Absidia orchidis (Vuill.) Hagem, a microorganism known to hydroxylate progesterone (II) at 6β, 7α, and 11α, and Reichstein’s substance S (III) at 6β, 11α, and 11β positions. In order to investigate the relationships between the structure of substrate molecules and the positions to be hydroxylated, we carried out the transformation of 3β,14,21-trihydroxy-14β-pregn-20-ene-3-one (IVA) and 4,5-dehydrodigitoxigenone (V) using this microorganism with the results that the former compound is hydroxylated at 1β and the latter mainly at 7β and 12β positions.

A. orchidis was grown for 66 hours with shaking on a nutrient medium containing glucose, peptone and corn steep liquor, the mycelium harvested, washed and suspended in distilled water. The substrate (IVb) dissolved in methanol was added to this mycelium suspension and incubation was continued for a further 48 hours. After usual treatment of the fermentation filtrate, a monohydroxylated product (VIA), C_{46}H_{54}O_{5}, m.p. 252~263°C, was obtained. As this substance gave a triacetate (VIB), C_{62}H_{60}O_{15}, m.p. 158~162°C, \([\alpha]_D^{25} +0.4^o\) (pyridine), the newly introduced hydroxyl was considered to be a secondary or a primary one, and proved to be at 1β-position on identification of VIB with ketol triacetate derived from acovenosigenin A (VII).

While the usual bioconversion performed by us of 4,5-dehydrodigitoxigenone (V) with A. orchidis produced several kinds of product, the use of suspensions of mycelium preincubated with progesterone gave one of them predominantly. This compound (IXa), C_{45}H_{52}O_{5}, m.p. 287~290°C, \([\alpha]_D^{25} +66.7^o\) (pyridine), UV: \(\lambda_{\text{max}}^{\text{UV}} 226 \text{ m}\mu \) (log \(\varepsilon 4.32\)), gave a diacetate (IXb) C_{52}H_{62}O_{10}, m.p. 267~268°C, \([\alpha]_D^{25} +52.1^o\) (pyridine), showing that both of the newly introduced hydroxyls are secondary or primary. Treatment of IXa with 1% hydrochloric acid in acetone afforded a 4"-5-ketone (X), C_{46}H_{58}O_{6}, m.p. 279~283°C, UV: \(\lambda_{\text{max}}^{\text{UV}} 283 \text{ m}\mu \) (log \(\varepsilon 4.40\)). This fact and the unsuccessful reduction of IXa with zinc dust in acetic acid indicated that one of the hydroxyls introduced into ring B was at 7-position.

*1 The appearance of absorption band at this wave length is due to overlapping of an absorption for \(\alpha,\beta\)-unsaturated ketone at 241 m\(\mu\) and that of unsaturated lactone at 217 m\(\mu\).