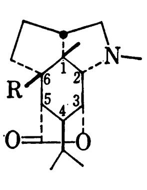
Communications to the Editor

[Chem. Pharm. Bull. 20(2) 418—421 (1972)]

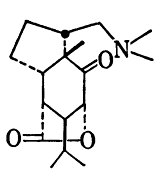
UDC 547.94.02 : 581.192

Further Studies on the Alkaloidal Constituents of *Dendrobium nobile* (Orchidaceae)—Structure Determination of 4-Hydroxy-dendroxine and Nobilomethylene

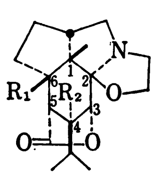
During the course of studies on the alkaloidal constituents of orchidaceous plant, *Dendrobium nobile* Lindl.,[1] it has been found that these consist of dendrobine[2] (Ia), nobilonine[3] (II), dendroxine[4] (IIIa), 6-hydroxy-dendrobine[5] (dendramine[6]; Ib), and 6-hydroxy-dendroxine[5] (IIIb). The structure of these alkaloids has been thoroughly investigated[2-9] by chemical and spectroscopic means, and especially dendrobine (Ia) and nobilonine (II), two main alkaloids of this plant, have been correlated by a mutual interconversion.[3]



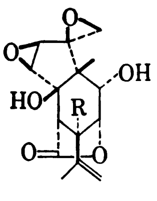
Ia : R=H
Ib : R=OH



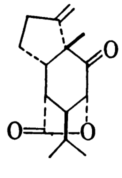
II



IIIa : $R_1=R_2=H$
IIIb : $R_1=OH$, $R_2=H$
IIIc : $R_1=H$, $R_2=OH$



IVa : R=H
IVb : R=OH



V

Gas chromatographic (GC) analysis of the crude basic principles of this plant revealed the presence of ten of substances, five of which have already been fully characterized (see Fig. 1). This report concerns the structure elucidation of the two minor components, *i.e.*, 4-hydroxy-dendroxine (IIIc) and nobilomethylene (V), which remained unclarified.

The pure material corresponding to the 9th peak of GC eluent was obtained as white plates of mp 208—210° after repeated chromatography in a yield of 15 mg from 82 kg of fresh bulbs (stems) of *Dendrobium nobile*. Its elemental composition was $C_{17}H_{25}O_4N$[10]$\cdot 1/2H_2O$ from combustion analysis[11] (Found: C, 63.92; H, 8.10; N, 4.43. Calcd.: C, 64.53; H, 8.28; N, 4.43) and may presumably be a hydroxylated dendroxine isomeric to 6-hydroxy-dendroxine (IIIb). The presence of hydroxyl group (ν_{OH}^{KBr} 3530 cm^{-1}) and five-membered ring lactone

---

[1] 高貴石斛 (Koki-sekkoku); cultivated one for ornamental purposes.

[2] T. Onaka, S. Kamata, T. Maeda, Y. Kawazoe, M. Natsume, T. Okamoto, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **12**, 506 (1964).

[3] T. Onaka, S. Kamata, T. Maeda, Y. Kawazoe, M. Natsume, T. Okamoto, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **13**, 745 (1965).

[4] T. Okamoto, M. Natsume, T. Onaka, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **14**, 672 (1966).

[5] T. Okamoto, M. Natsume, T. Onaka, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **14**, 676 (1966).

[6] Y. Inubushi, Y. Tsuda, and E. Katarao, *Chem. Pharm. Bull.* (Tokyo), **14**, 668 (1966).

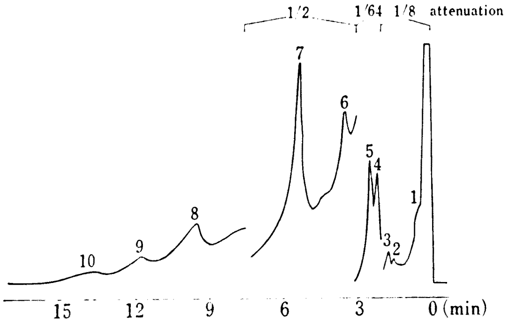
[7] For review, see L.A. Porter, *Chem. Rev.*, **67**, 454 (1967).

[8] Y. Inubushi, Y. Sasaki, Y. Tsuda, B. Yasui, T. Konita, J. Matsumoto, E. Katarao, and J. Nakano, *Yakugaku Zasshi*, **83**, 1184 (1963); *idem*, *Tetrahedron*, **20**, 2007 (1964).

[9] S. Yamamura and Y. Hirata, *Tetrahedron Letters*, **1964**, 79; *idem*, *Nippon Kagaku Zasshi*, **85**, 377 (1964).

[10] The elemental composition was confirmed by exact mass measurement of high resolution mass spectrum.

[11] F & M GC Model 180 CHN Analyzer was used for the determination.



Column 1.5% QF 1 1.5m at 208°

Fig. 1

peak No.	substance
1	unknown
2	nobilomethylene (V)
3	neutral substance
4	dendrobine (Ia)
5	nobilonine (II)
6	6-hydroxy-dendrobine (dendramine; Ib)
7	dendroxine (IIIa)
8	6-hydroxy-dendroxine (IIIb)
9	4-hydroxy-dendroxine (IIIc)
10	neutral substance

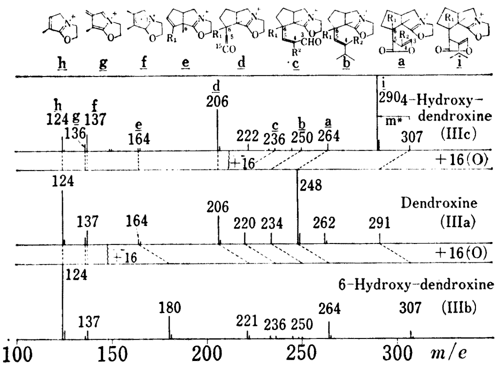


Fig. 2

TABLE I

δ (CDCl$_3$) 100 Mc	$-CH\langle^{Me}_{Me}$	t-Me	$-N\langle^{CH_2CH}_{CH_2CH_2O}$	$CH\backslash$ CHCOO $C/$	NCH$_2$CH$_2$O	COOCH$\langle^{CH}_{C}$
Dendroxine[a]	0.87, 0.95 (d,d, $J=6.0$)	1.16	2.7—3.2 (m)		3.80 (t, $J=6.0$)	4.11 (d, $J=3.4$)
6-Hydroxy-dendroxine	1.02 (d, $J=6.3$)	1.16	2.6—3.44 (m)	2.74 (d, $J=3.5$)	3.66—4.06 (m)	4.38 (d, $J=4.5$)
4-Hydroxy-dendroxine	0.98 (t, $J=6.1$)	1.25	2.74—3.37 (m)	2.45 (d, $J=4.7$)	3.69—4.00 (m)	4.08 (s)

[a] Measured at 60Mc.

(ν_{CO}^{KBr} 1783 cm^{-1}) is indicated from its infrared (IR) spectrum, and its nuclear magnetic resonance (NMR) spectrum reveals the presence of an oxazolidine ring similar to dendroxine (IIIa) and 6-hydroxy-dendroxine (IIIb) in addition to the presence of functional groups listed in Table I. This minor alkaloid exhibits a singlet attributable to the methine proton adjacent to the lactone oxygen in contrast to the doublet in dendroxine (IIIa) or the quartet in dendrobine (Ia). As there is no additional signal for the hydrogen on the carbon bearing oxygen, the hydroxyl group of this molecule must exist as a tertiary hydroxyl group.

The mass spectrum of this new alkaloid is similar to those of dendroxine (IIIa) and 6-hydroxy-dendroxine (IIIb), again suggesting the isomeric character of this alkaloid with IIIb. Thus, as indicated in Fig. 2, the main fragments patern below *m/e* 206 is superimposable to that of dendroxine (IIIa), while increments by 16 mass (corresponding to oxygen) are observed in the peaks above *m/e* 222 up to M$^+$ ion at *m/e* 307. Taking account of the fragmentation mechanism established in our earlier study,[5] these fragment ions can be formulated by the structure shown in Fig. 2. The position of attachment of the additional hydroxyl group of this alkaloid may be deduced by the consideration of the structure of the fragments in combination with the fact that the fragments e, f, and g no longer bear additional oxygen, whereas a, b, and c still retain the oxygen. More particularly, the oxygen in question will exist on the carbon atom contained in all of the fragments a, b, and c but not in e, f, and g.

Such carbons are C-4 and C-5, then this alkaloid should be 4- or 5-hydroxy-dendroxine. Of these two possibilities, the former is more probable when one recalls the singlet nature of C-3 methine signal in the NMR spectrum of this compound, and hence IIIc (4-hydroxy-dendroxine) is proposed for this new minor alkaloid of *Dendrobium nobile*. The remarkable feature of the mass spectrum of 4-hydroxy-dendroxine (IIIc) is the appearance of M-17 (OH) peak (fragment ion i) as the most intense peak. This would provide another proof of the presence of hydroxyl group at the C-4 position, when one considers the similar easy cleavage of isopropyl group at the same C-4 position to give the ion a in the mass spectra of the series of these alkaloids. The conclusive evidence for the structure (IIIc) is the presence of C-5 methine signal as a narrow doublet at δ 2.45 ($J_{5,6}=4.7$) in the NMR spectrum of 4-hydroxy-dendroxine. The corresponding C-5 methine proton signals have been observed at δ 2.68 ($J_{4,5}=3.8$) and δ 2.74 ($J_{4,5}=3.5$) in the NMR spectra of 6-hydroxy-dendrobine (Ib)[5,6] and 6-hydroxy-dendroxine (IIIb)[5] in agreement with the above assignment.

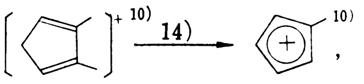
Finally the stereochemistry at C-4 of 4-hydroxy-dendroxine is assumed as IIIc because of the structural relationship to other dendrobium alkaloids. In this connection, it will be interesting to note that the same type of biological C-4 hydroxylation of tutin (IVa) has been reported to give mellitoxine[12] (hyenandin[13]; IVb).

The other component which was obtained for the first time by the present study (3 mg from 82 kg of fresh bulb) turned out to be a neutral principle although it was isolated from a basic portion of the extract of the plant. This neutral substance, colorless plates from hexane, mp 159.5—160.5°, corresponds to the second peak of the gas chromatogram in Fig. 1 and its elemental composition was $C_{15}H_{20}O_3$ from high resolution mass spectrometry. Its IR spectral characteristics indicate the partial structure of γ-lactone ($\nu_{\mathrm{CO}}^{\mathrm{KBr}}$ 1784 cm^{-1}), 6-membered ring ketone ($\nu_{\mathrm{CO}}^{\mathrm{KBr}}$ 1726 cm^{-1}), and exocyclic methylene ($\nu_{\mathrm{C=CH_2}}^{\mathrm{KBr}}$ 3070, $\nu_{\mathrm{C=C}}^{\mathrm{KBr}}$ 1648, and $\delta_{\mathrm{C\text{-}CH_2}}^{\mathrm{KBr}}$ 898 cm^{-1}). NMR spectrum of this compound shows the presence of several functional groups identical to nobilonine (II), while two distinct differences (*i.e.*, the presence of exocyclic methylene and the absence of dimethylamino grouping) are also observed (see Table II).

TABLE II

	$-\mathrm{CH}{<}^{\underline{\mathrm{Me}}}_{\underline{\mathrm{Me}}}$	t-$\underline{\mathrm{Me}}$	$\mathrm{NMe_2}$	$-\mathrm{COO{-}C\underline{H}}{<}^{\mathrm{CH}}_{\mathrm{C}}$	$^{-\mathrm{CH_2}}_{\mathrm{C}}{>}\mathrm{C{=}C\underline{H}_2}$
Nobilonine	0.93 (d, $J=6.0$) 0.98 (d, $J=6.0$)	1.35	2.10	4.56 (d, $J=5.6$)	—
Nobilomethylene	0.94 (t, $J=6.2$)	1.24	—	4.50 (d, $J=5.5$)	4.70 (t, $J=3.3$) 4.87 (t, $J=2.6$)

These spectral evidences indicate a nobilonine skeleton for this compound and hence the name of nobilomethylene is proposed. A close similarity between nobilomethylene and nobilonine is also observed in their mass spectra. In the spectrum of nobilomethylene a fragmentation process of $\mathrm{M}^+ \xrightarrow{14)} \text{M-43(iso-Pr)}^+ \xrightarrow{14)} \text{M-43-44(CO}_2\text{)}^+$ was proved by the presence of appropriate metastable ions, and this process is completely analogous with the behavior of nobilonine (II) upon electron impact. Another equally important fragmentation is

$\mathrm{M}^+ \xrightarrow{14)} \text{M-28(CO)}^+ \xrightarrow{14)} \text{M-28-43(iso-Pr)}^+ \xrightarrow{14)}$ 

, and this pro-

---

12) R. Hodges, E.P. White, and J.S. Shannon, *Tetrahedron Letters*, **1964**, 371.

13) G. Jommi, P. Manitto, F. Pelizzoni, and C. Scolastico, *Chim. Ind.* (*Milan*), **46**, 549 (1964); **47**, 406 (1965); **47**, 407 (1965).

14) The presence of metastable ion at appropriate position confirms this fragmentation process.

cess is also substantially analogous to that of dendrobine (Ia) and dendroxine (IIIa) discussed earlier.[5] On these grounds, V would represent the structure of nobilomethylene, and chemical confirmation of this proposal was next investigated and achieved.

After nobilonine (II) was oxidized with 30% H_2O_2 in EtOH, the crude N-oxide was heated at 150—200° in vacuum, and the sublimed product was purified by recrystallization. The identity of this product with the specimen of nobilomethylene was confirmed by IR, mixed mp determination, and optical rotatory dispertion (ORD) comparisons.

It should be of interest to give some comments about the occurrence of nobilomethylene (V) in the basic portion of the extract of this orchid. We are of the opinion that nobilomethylene would be an artifact and the precursor of this compound is nobilonine N-oxide whose pyrolysis has been proved to give nobilomethylene (V) by the above experiment. The suggestive, although not conclusive, evidences for the above presumption are as follows: (i) Nobilomethylene was not obtained from the neutral portion of the extract in spite of careful examination of the chromatographic fractions by the absorption of γ-lactone region in the IR spectra. (ii) Some chromatographic fractions of the basic portion of the extract furnished very polar and gummy materials which showed a behavior similar to nobilonine N-oxide on a thin-layer chromatography (TLC) plate. Purification of these fractions was not possible owing to their highly polar character. (iii) These chromatographic fractions show a peak corresponding to nobilomethylene (V) on GC analysis although the fractions do not contain any nobilomethylene (V) by TLC analysis (pyrolysis must occur at evaporating chamber of the gas chromatograph).

Acknowledgement The authors are grateful to Dr. J. Shinoda and Dr. T. Ishiguro for their kind encouragements.

Faculty of Pharmaceutical Sciences,
University of Tokyo
Hongo, Tokyo

TOSHIHIKO OKAMOTO
MITSUTAKA NATSUME[15]
TADAMASA ONAKA[15]

Central Research Laboratory,
Daiichi Seiyaku Co., Ltd.
Minami-funabori-cho, Edogawa-ku, Tokyo

FUMIHIKO UCHIMARU
MASAO SHIMIZU

Received November 19, 1971

---

[15] Present address: *Itsuu Laboratory, Tamagawa 2-28-10, Setagaya-ku, Tokyo.*