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DENDROBINE AND 3-HYDROXY-2-OXODENDROBINE FROM *DENDROBIUM NOBILE*[1]

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ABSTRACT.—Dendrobine (**1**) and a new alkaloid, 3-hydroxy-2-oxodendrobine (**2**), were isolated from the fresh stems of *Dendrobium nobile*, and their structures were elucidated on the basis of spectral evidence and direct comparison with authentic samples. The nmr spectral properties of these compounds were also studied.

Dendrobium nobile Lindl. (Orchidaceae), known in China as "Chin-Chai-Shi-Hu," is used as a tonic in traditional medicine. The stems of the plant, fresh or processed, are prescribed to improve appetite, stimulate salivary secretion, and promote general health (1,2). The herb is frequently taken by opera singers to improve their voices. Prior phytochemical work on *D. nobile* has resulted in the isolation of more than ten alkaloids of the dendrobane-type (3-10), as well as some nonalkaloidal constituents such as sesquiterpenes, triterpenes, and a quinone (9,11). Pharmacologically, the dendrobane-type alkaloids have been shown to possess similar activities as the structurally related picrotoxinin (12).

In the present paper, we report the isolation and characterization of two alkaloids, dendrobine (**1**), a known constituent of *Dendrobium* spp. (13-16), and a new compound **2**, to which we have assigned a structure of 3-hydroxy-2-oxodendrobine.[2] The high-field and two-dimensional nmr spectral characteristics of these compounds are also presented.

RESULTS AND DISCUSSION

Dendrobine (**1**) was isolated as colorless needles, mp 135-136°, $[\alpha]_D$ −46.8 (EtOH), displaying a molecular ion at *m/z* 263.1880, consistent with a molecular formula of $C_{16}H_{25}O_2N$ (calcd. value 263.1885. The observed physical and spectral properties of the isolate were in close agreement with the literature values for dendrobine (13-15). Its identity was confirmed by comparison with a reference sample (mmp, tlc, ms).

Partial assignments of the low-field ^{1}H-nmr spectra of dendrobine and related compounds have been reported previously (4-10, 13-19). However, with increasing interest in synthesis and structure-activity relationship studies (12, 20-24), detailed knowledge of the ^{1}H-nmr and ^{13}C-nmr spectral properties of dendrobine and its derivatives will be helpful. In this report, complete assignment of the 360 MHz ^{1}H-nmr and the ^{13}C-nmr data for both dendrobine and 3-hydroxy-2-oxodendrobine are presented in Table 1.

In the ^{1}H-nmr spectrum, four methyl signals for **1** were identified and assigned on the basis of literature data (24). Further downfield, the salient features are a doublet of doublets at δ 4.83, representing the H-9 proton, and a doublet at δ 2.68 due to H-10.

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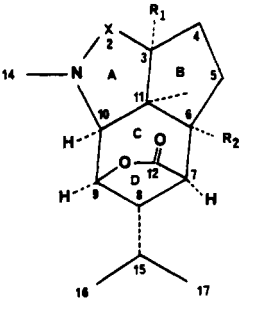
[2]In the literature, several numbering systems have been used for the dendrobane nucleus. In the present study, the IUPAC numbering system was adopted.

TABLE 1. ^{1}H-nmr and ^{13}C-nmr Chemical Shifts of Dendrobine (**1**) and 3-Hydroxy-2-oxodendrobine (**2**)

^{1}H nmr (360 MHz, $CDCl_3$)			^{13}C nmr (90.8 MHz, $CDCl_3$)		
Proton	**1**	**2**	Carbon	**1**	**2**
H-2a	3.22 t (J=9)	—	C-2	61.9	174.6
H-2b	2.70 t (J=9)	—			
H-3	2.35 pentet (J=9)	—	C-3	51.4	86.0
H-4a	1.54	2.02	C-4	32.7	38.0
H-4b	1.85	2.25			
H-5a	2.06	1.84	C-5	30.7	29.2
H-5b	2.15	2.05			
H-6	2.01	2.27	C-6	43.8[a]	43.8[a]
H-7	2.45 dd (J=4, 9.5)	2.53 dd (J=4.5, 5.5)	C-7	53.5	50.8
H-8	2.11	2.25	C-8	42.9[a]	43.0[a]
H-9	4.83 dd (J=3, 5.5)	4.73 dd (J=3, 5.5)	C-9	79.0	75.4
H-10	2.68 d (J=3)	3.18 d (J=3.5)	C-10	66.7	64.3
			C-11	52.3	47.3
			C-12	178.9	177.6
14-Me	2.50 s	2.88 s	C-14	36.4	25.2
H-15	1.75 d of heptet (J=6, 6.5)	2.07	C-15	24.4	24.5
16-Me	0.95 d (J=6.5)	1.03 d (J=6.5)	C-16	20.8[b]	20.5[b]
17-Me	0.96 d (J=6.5)	1.04 d (J=6.5)	C-17	21.0[b]	21.2[b]
18-Me	1.38 s	1.37 s	C-18	32.6	27.8
OH	—	3.43 bs			

[a,b]Assignment may be interchanged.

These assignments are in general agreement with literature values (13-19) and were confirmed by the COSY spectrum (Figure 1a). Furthermore, the spectrum reveals some proton signals which were not well resolved at low field. Thus, it was possible to assign a doublet of doublets at δ 2.45 to H-7, and a pentet at δ 2.35 to H-3. Other signals were analyzed through interpretation of the 2-D COSY spectrum. It is clear that H-9 couples to, besides H-10, a signal hidden under an envelope at ca. δ 2.11. This signal was, therefore, assigned to H-8. On the other hand, H-7 displays couplings with both H-8 and part of the same envelope at ca. δ 2.01, which must be due to H-6. The isopropyl methyl signals (16- and 17-Me) exhibit couplings with part of a diffused multiplet centered at δ 1.75. Detailed examination indicated that this signal is a doublet of heptets, attributed to H-15. The rest of the signals in the spectrum are then due to the methylene groups. Thus, a triplet at δ 3.22 was assigned to a proton at C-2 (H-2a),



1 X=CH_2, R_1=R_2=H
2 X=C=O, R_1=OH, R_2=H
3 X=CH_2, R_1=H, R_2=OH
4 X=C=O, R_1=H, R_2=OH

which displays strong coupling with another triplet at δ 2.70 (H-2b). The coupling information of H-3 (δ 2.35) permits assignments to be made for H-4a (δ 1.54) and H-4b (δ 1.85). Finally, the two signals hidden under an envelope at δ 2.06 and 2.15 were assigned to the 5-CH_2 protons.

The ^{13}C-nmr spectrum of **1** (Table 1) displays all sixteen carbons. The C-12 could be readily identified at the carbonyl region (δ 178.9). All other carbons resonating between 20 and 80 ppm were differentiated with the aid of attached proton technique (APT) and the SFORD spectra. Thus, the only quaternary carbon signal in the spectrum (δ 52.3) was assigned to C-11, an oxygen-bearing CH signal (δ 79.0) to C-9, and two deshielded CH signals (δ 53.5 and 66.7) to C-7 and C-10, respectively. Among the three methylene signals, the 2-CH_2 was assigned to a peak at δ 61.9, and the 4- and 5-CH_2 to 32.7 and 30.7, respectively. Of the four methyl groups, the 16- and 17-Me resonate at upfield (δ 20.8 and 21.0), while the 14-Me resonates at δ 36.4, and the 18-Me at δ 32.6. Other methine carbons (C-3, C-6, C-8, and C-15) were determined with reference to compound **2** described below.

3-Hydroxy-2-oxodendrobine (**2**), isolated as colorless needles, mp 210-211°, $[\alpha]_D$ −52.7 (EtOH), revealed a molecular ion at *m/z* 293.1645, analyzing for $C_{16}H_{23}O_4N$ (calcd. value 293.1627). A structure closely related to dendrobine was suggested when their nmr spectra were compared. In the ^{1}H-nmr spectrum of **2** (Table 1), downfield shifts of the *N*-Me and the H-10 peaks, together with the absence of the 2-CH_2 signals, indicated the presence of a lactam structure. Similar changes have been observed when dendramine (**3**) was oxidized to oxodendramine (**4**) (6). Compound **2** was therefore considered to possess a carbonyl group at C-2. In support of this, the ir absorptions (υ max 1676, 1693 cm^{-1}) and a carbonyl carbon signal at δ 174.6 (in addition to the lactone signal at 177.6) were diagnostic for a lactam ring. As expected, the *N*-Me carbon is shifted upfield by ca. 10 ppm.

Both the ir spectrum (υ max 3335-3375 cm^{-1}) and the ^{1}H-nmr spectrum (δ 3.43,

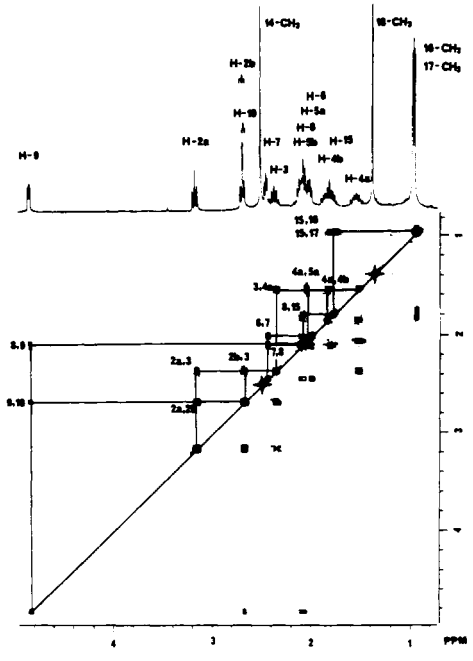


FIGURE 1. ^{1}H-^{1}H homonuclear correlation spectrum of dendrobine (**1**).

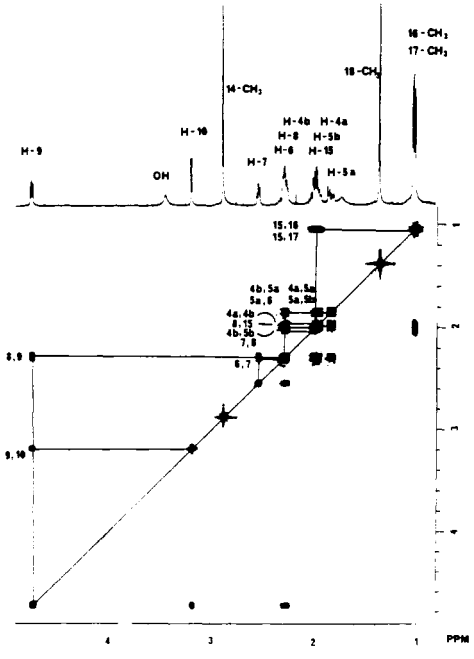


FIGURE 2. ^{1}H-^{1}H homonuclear correlation spectrum of 3-hydroxy-2-oxo-dendrobine (**2**).

bs, D_2O exchangeable) revealed the presence of a hydroxyl group in the molecule. With the observation of an oxygen-bearing quaternary carbon signal (δ 86.0), it became apparent that the hydroxyl group was tertiary. Assignment of this hydroxyl function was then derived from the following evidence.

Examination of the mass spectra of **1** and **2** (Table 2) revealed several common fragmentation characteristics of dendrobanes as proposed previously (6,8). Thus, compound **2** exhibited a fragmentation behavior which was analogous to that of **1** in many respects. However, ions from **2** were shifted by 30 mass units when compared with **1**, indicating that both the lactam and the hydroxyl groups are located in rings A/B. In particular, an ion peak observed at *m/z* 126 (M^+-167, $C_6H_8O_2N$), corresponding to an ion containing ring-A only, was shown to bear two oxygens. Therefore, assignment of the hydroxyl group was limited to either the C-3 or C-10 positions. The final decision was made on the basis of the ^{1}H-nmr data (Figure 2).

TABLE 2. Mass Spectra of Dendrobine (**1**) and 3-Hydroxy-2-oxodendrobine (**2**)

Ion	1	2
M^+	263 (14%)	293 (21%)
M^+-15	248 (1)	278 (7)
M^+-28	235 (2)	265 (4)
M^+-43	220 (55)	250 (2)
M^+-57	206 (6)	236 (1)
M^+-71	192 (6)	222 (3)
M^+-85	178 (8)	208 (5)
M^+-99	164 (6)	194 (4)
M^+-127	136 (13)	166 (2)
M^+-154	109 (16)	139 (2)
M^+-155	108 (20)	
M^+-167	96 (100)	126 (20)
		127 (100)
	81 (10)	81 (8)

The most downfield proton of **2** is the H-9 (δ 4.73) which typically appears as a doublet of doublets. Thus, the COSY spectrum permits unambiguous assignments of H-10 (δ 3.18) and H-8 (δ 2.25). The fact that H-9 appears as a doublet of doublets further confirms the presence of a proton at C-10. Consequently, the hydroxyl group can only be placed at C-3. In this manner, all other features of the ^{1}H-nmr spectra can be interpreted by the proposed structure **2**.

The ^{13}C-nmr spectral properties of **2** resemble **1** in many respects. Some differences were observed in the ring-A carbons, as a result of the introduction of a lactam and a hydroxyl functional group. Comparison of the methine carbon signals of **1** and **2** permits the assignments of C-3, C-6, C-8, and C-15. As shown in Table 1, C-15 is invariably the most shielded carbon (ca. δ 24.5), and C-6 and C-8 are close to each other (δ 43-44). On the other hand, C-3 (δ 51.4 in **1**) is deshielded in **2** by the effects of the hydroxyl and lactam functionalities and becomes quaternary.

Compound **2** is, therefore, determined to be 3-hydroxy-2-oxodendrobine. The stereochemistry of this compound is considered to be the same as in dendrobine and other related compounds (17,25) on the basis of chemical shifts and coupling constants of the protons. 3-Hydroxy-2-oxodendrobine is isomeric to oxodendramine (**4**), which has a hydroxy at C-6 (6). Chemically, it represents an oxidized form of dendrobine. Although it has been demonstrated that dendrobine could be oxidized to 2-oxodendrobine by treatment with potassium permanganate-magnesium sulfate (13), we be-

lieve that **2** is a natural product since it was isolated from the plant extract without any pretreatment with oxidizing agents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Specific rotations were measured on a Perkin-Elmer 241 Polarimeter. The uv spectra were obtained with a Beckman DU-7 Spectrophotometer, and ir spectra on a Nicolet MX-1 FT-IR Spectrophotometer. Nmr data were collected on a Nicolet NMC-360 instrument, operating at 360 MHz for proton resonance and 90.8 MHz for carbon resonance. TMS was used as an internal standard, and chemical shifts were expressed in δ (ppm). Hrms were recorded on a ZAB-2F VG Analytical Organic Mass Spectrophotometer.

PLANT MATERIAL.—*D. nobile* was collected before flowering in Shih-Mein, Sichuan, China. Voucher specimens have been deposited at the West China Medical University.

EXTRACTION AND FRACTIONATION.—Fresh stems of *D. nobile* (28.5 kg) were extracted with 95% EtOH to produce 870 g of extractive. The alcoholic extract was then dissolved in 5% HCl solution and washed with Et_2O. It was made alkaline (pH 10) by adding Na_2CO_3, followed by $CHCl_3$ extractions. The acid-base extraction procedure was repeated once to give a crude alkaloid fraction.

The crude alkaloid fraction was then chromatographed on alumina preparative tlc plates, using a solvent system containing $CHCl_3$-Et_2O-EtOAc (20:20:1). Treatment with iodine vapor revealed a band at Rf 0.7-0.75. Usual workup on this band afforded a white powder, which exhibited two spots (Rf 0.38 and 0.15) on tlc (Si gel, 10% MeOH in EtOAc).

ISOLATION OF 3-HYDROXY-2-OXODENDROBINE (**2**).—The white powder was dissolved in Et_2O and left overnight. Colorless fine needles were formed and recrystallized from the same solvent to afford 25 mg of **2**, mp 210-211°; $[\alpha]^{20}D$ −52.7 (EtOH, c 0.3); uv (EtOH) λ max 209 nm (log ε 3.36); ir (KBr) υ max 3375-3335, 2967, 2940, 2870, 1785, 1776, 1693, 1676 cm^{-1}; ^1H nmr ($CDCl_3$, 360 MHz), see Table 1; ^{13}C nmr ($CDCl_3$, 90.8 MHz), see Table 1; ms, see Table 2.

ISOLATION OF DENDROBINE (**1**).—The mother liquor of **2** contained a single spot (Rf 0.15) on tlc (10% MeOH in EtOAc). It was dried in vacuo, redissolved in petroleum ether, and stored in a freezer for 2 days. Colorless crystals were obtained after filtration and recrystallized twice from Et_2O to afford a colorless compound **1** (326 mg), mp 135-136°; $[\alpha]^{20}D$ −46.8 (EtOH, c 1.0); uv (EtOH) λ max 213 nm (log ε 2.85); ir (KBr) υ max 1765 cm^{-1}; ^1H nmr ($CDCl_3$, 360 MHz), see Table 1; ^{13}C nmr ($CDCl_3$, 90.8 MHz), see Table 1; ms, see Table 2.

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LITERATURE CITED

1. *China's Pharmacopoeia*, Part One, The People's Health Sciences Publication C:., Beijing (Peking), China, 1977, p. 145.
2. Jiangsu Medical College (ed.), *A Dictionary of Chinese Materia Medica*, Shanghai Scientific Technology Press, Shanghai, China, 1977, p. 586.
3. Y. Inubushi, H. Ishii, B. Yasui, T. Konita, and T. Harayama, *Chem. Pharm. Bull.*, **1**., 1175 (1964).
4. T. Onaka, S. Kamata, T. Maeda, Y. Kawazoe, M. Natsume, T. Okamoto, F. Uchi u, and M. Shimisu, *Chem. Pharm. Bull.*, **13**, 745 (1965).
5. Y. Inubushi and J. Nakano, *Tetrahedron Lett.*, 2723 (1965).
6. Y. Inubushi, Y. Tsuda, and E. Kararao, *Chem. Pharm. Bull.*, **14**, 668 (1966).
7. T. Okamoto, M. Natsume, T. Onaka, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.*, **14**, 672 (1966).
8. T. Okamoto, M. Natsume, T. Onaka, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.*, **14**, 676 (1966).

9. T. Okamoto, M. Natsume, T. Onaka, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.*, **20**, 418 (1972).
10. K. Hedman and K. Leander, *Acta Chem. Scand.*, **26**, 3177 (1972).
11. B. Talapatra, P. Mukhopadhyay, P. Chaudhury, and S.K. Talapatra, *Indian J. Chem.*, **21B**, 386 (1982).
12. L.A. Porter, *Chem. Rev.*, **67**, 441 (1967).
13. Y. Inubushi, Y. Sadaki, Y. Tsuda, B. Yasui, T. Konita, J. Matsumoto, E. Katarao, and J. Nakano, *Tetrahedron*, **20**, 2007 (1964).
14. T. Onaka, S. Kamata, T. Maeda, Y. Kawazoe, M. Natsume, T. Okamoto, F. Uchimaru, and M. Shimizu, *Chem. Pharm. Bull.*, **12**, 506 (1964).
15. S. Yamamura and Y. Hirata, *Tetrahedron Lett.*, 79 (1964).
16. I. Granelli, K. Leander, and B. Luning, *Acta Chem. Scand.*, **24**, 1209 (1970).
17. D. Behr and K. Leander, *Acta Chem. Scand.*, **26**, 3196 (1972).
18. M. Elander and K. Leander, *Acta Chem. Scand.*, **25**, 717 (1971).
19. K. Hedman, K. Leander, and B. Luning, *Acta Chem. Scand.*, **25**, 1142 (1971).
20. K. Yamada, M. Suzuki, Y. Hayakawa, K. Aoki, H. Nakamura, H. Nagase, and Y. Hirata, *J. Am. Chem. Soc.*, **94**, 8278 (1972).
21. Y. Inubushi, T. Kikuchi, T. Ibuka, K. Tanaka, I. Saji, and K. Tokane, *J. Chem. Soc., Chem. Comm.*, 1252 (1972).
22. Y. Inubushi, T. Kikuchi, T. Ibuka, K. Tanaka, I. Saji, and K. Tokane, *Chem. Pharm. Bull.*, **22**, 349 (1974).
23. W.R. Roush, *J. Am. Chem. Soc.*, **100**, 3599 (1978).
24. W.R. Roush, *J. Am. Chem. Soc.*, **102**, 1390 (1980).
25. I. Granelli and K. Leander, *Acta Chem. Scand.*, **24**, 1108 (1970).

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