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Phenanthrenes from *Dendrobium plicatile*[☆]

Chie Honda, Masae Yamaki*

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663-8179, Japan

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Abstract

From *Dendrobium plicatile* stems, three phenanthrenes were isolated. The structures are 2,5-dihydroxy-4,9,10-trimethoxyphenanthrene, 2,5-dihydroxy-4-methoxyphenanthrene and 2,5,9-trihydroxy-4-methoxy-9,10-dihydrophenanthrene.

Keywords: Dendrobium plicatile; Orchidaceae; Phenanthrene; Plicatol A, B and C

1. Introduction

In the course of our investigations on the constituents of *Dendrobium plicatile*, we have already reported on the isolation and characterization of seven stilbenoids (Yamaki & Honda, 1996). In this paper, we describe the structural elucidation of three phenanthrene derivatives isolated from the stems of this plant.

2. Results and discussion

The ethyl acetate soluble portion of the methanolic extract of *Dendrobium plicatile* was repeatedly chromatographed over silica gel to give three phenanthrene derivatives, named as plicatol A (**1**), B (**2**) and C (**3**).

Plicatol A (**1**) showed UV absorption maxima at 260, 280 and 320 nm similar to other phenanthrene derivatives. The IR spectrum exhibited absorptions at 3592 and 3267 (OH) (Fisch, Flick & Arditti, 1973), and at 1614, 1539 and 1334 cm^{-1} (aromatic rings). The mass spectrum of **1** displayed a molecular ion peak at m/z 300 $[\text{M}^+]$ ($C_{17}H_{16}O_5$). The ^{1}H- NMR spectrum showed signals for three adjacent aromatic protons (δ 7.83, *dd*, J = 8.1, 1.3 Hz, H-8; 7.51, *dd*, J = 7.7, 8.1 Hz, H-7; 7.20, *dd*, J = 7.7, 1.3 Hz, H-6), a pair of *meta*-coupled aromatic protons (δ 7.39, *d*, J = 2.6 Hz, H-1; 6.79, *d*, J = 2.6 Hz, H-3), two hydroxyl protons (δ 9.43, 5-OH; 5.73, 2-OH) and three methoxyl groups (δ 4.02, 4.03 and 3.99). However, signals due to *ortho*-coupled H-9 and H-10 protons of phenanthrene derivatives were absent, suggesting that **1** has substituents at C-9 and/or C-10. NOE enhancement was used to determine the assignment of signals and the position of the functional groups. Irradiation of the methoxyl signal at δ 3.99 gave an NOE at δ 7.39 (2.3%, H-1) and irradiation of the methoxyl signal at δ 4.03 increased the intensity of the ^{1}H signal at δ 7.83 (1.8%, H-8). Thus, the two methoxyl groups were considered to be located at C-9 and C-10, and the signals at δ 4.03 and 3.99 were then assigned to 9-OMe and 10-OMe, respectively. When the resonances at δ 4.02 (4-OCH_3) and 6.79 (H-3) were irradiated, the latter increased the intensity of the methoxyl signal at 4.02 (8.2%, 4-OCH_3), while the former showed NOEs to protons at δ 6.79 (7.4%, H-3) and 9.43 (2.4%, 5-OH). Therefore, it was reasonable to assume that the remaining methoxyl group was located at C-4 and two hydroxyl groups were at C-2 and C-5. Accordingly, the structure of **1** was established as 2,5-dihydroxy-4,9,10-trimethoxyphenanthrene. This was supported by the HMQC and HMBC spectra (Fig. 1).

[☆] Part 2

* Corresponding author.

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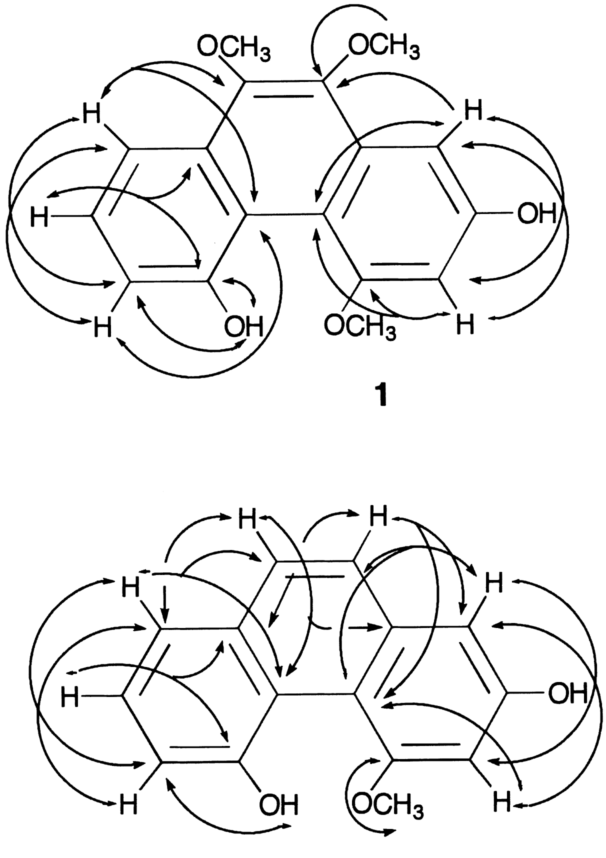
Plicatol B (**2**) showed UV absorption maxima at 255, 285 and 315 nm. The IR spectrum exhibited absorptions at 3600, 3250 and 1620 cm^{-1}. The mass spectrum of **2** showed a molecular ion peak at *m*/*z* 240 [M$^+$] ($C_{15}H_{12}O_3$). The ^{1}H NMR spectrum showed signals due to three adjacent aromatic protons, a pair of *meta*-coupled aromatic protons, two hydroxyl protons and a methoxyl group (δ 4.04) along with signals due to a pair of *ortho*-coupled protons (δ 7.61, *d*, J = 9.0 Hz, and 7.41, *m*, overlapped with H-8) ascribable to H-9 and H-10 of a phenanthrene ring. The overlapping signals of H-8 and H-10 were clearly resolved in the ^{1}H NMR spectrum of the diacetate of **2**, prepared as described in Section 3. From these data, **2** was considered to be a phenanthrene derivative having a methoxyl and two hydroxyl groups. Locations of the methoxyl and hydroxyl groups in **2** were determined by NOE enhancement experiments. Irradiation of the methoxyl signal at δ 4.04 gave large NOEs at δ 9.64 (7.3%, 5-OH) and 6.83 (15.4%, H-3). In turn, irradiation of the ^{1}H signal at δ 6.83 (H-3) showed effects at δ 4.04 (5.2%, 4-OCH_3) and 5.68 (2.7%, 2-OH). These findings indicated that the methoxyl group was at C-4 and two hydroxyl groups were at C-2 and C-5. Thus, the structure of **2** was determined to be 2,5-dihydroxy-4-methoxyphenanthrene based on this data and on analysis of the HMQC and HMBC spectra (Fig. 1).

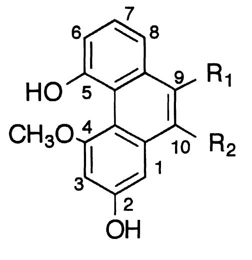
Plicatol C (**3**) showed UV absorption maxima at 272 and 304 nm. The IR spectrum exhibited absorptions at 3550, 3300, 1620 and 1460 cm^{-1}. The mass spectrum of **3** displayed a molecular ion peak at *m*/*z* 258 [M$^+$] ($C_{15}H_{14}O_4$). The ^{1}H- NMR spectrum showed signals due to three adjacent aromatic protons, a pair of *meta*-coupled aromatic protons and a methoxyl group, along with signals for three protons due to H-9 and H-10 of a dihydrophenanthrene ring (δ 4.58, 1H, *dd*, J = 4.0, 10.0 Hz, H-9ax; 2.74, 1H, *dd*, J = 10.0, 15.0 Hz, H-10ax and 2.96, 1H, *dd*, J = 4.0, 15.0 Hz, H-10eq). From these data, **3** was considered to be a dihydrophenanthrene derivative having a methoxyl and three hydroxyl groups. Locations of the methoxyl and hydroxyl groups in **3** were determined by NOE enhancement. Irradiation of the methoxyl signal at δ 3.93 gave a large NOE at δ 6.56 (10%, H-3). In turn, irradiation of the ^{1}H signal at δ 6.56 (H-3) affected only the signal of 4-OMe (7.9%). Irradiations at δ 7.09 (H-8) and at δ 2.96 (H-10) showed enhancements of the H-9 (3.6%) and H-1 (9%) signals, respectively. These findings indicated that a methoxyl group was at C-4 and three hydroxyl groups were at C-2, C-5 and C-9. Therefore, the structure of **3** was determined to be 2,5,9-trihydroxy-4-methoxy-9,10-dihydrophenanthrene. Phenanthrene derivatives with three adjacent protons on a C-ring are rare in nature. Biosynthetically these compounds may be derived from bibenzyl by *o*,*o*-oxidative coupling, as hircinol and loroglossol (Fisch et al., 1973) are derived from 3,3′,5-trihydroxybibenzyl (Gorham, 1995). Recently, three compounds, fimbriols A and B (Tezuka, Yoshida, Kikuchi & Xu, 1993) and 4,9-dimethoxy-2,5-dihydroxyphenanthrene (Leong, Kang, Harrison & Powell, 1997) have been reported.



1

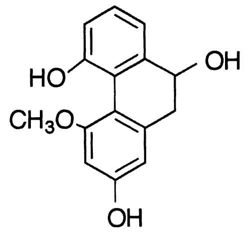
2

Fig. 1. HMBC correlations of **1** and **2**.



1 R_1=OCH_3, R_2=OCH_3

2 R_1= R_2=H



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3. Experimental

IR: $CHCl_3$; UV: MeOH; ^1H- and ^{13}C- NMR: 500 and 125 MHz, respectively, TMS as int. standard. MS: EIMS, 70 eV. CC and TLC were performed using Merck silica gel unless otherwise stated.

3.1. Plant materials

See Yamaki & Honda, 1996.

3.2. Extraction and isolation

The crude drug (20 kg) (stems of *D. plicatile*) was extracted with MeOH at room temperature. After evaporation of the solvent, the residue was diluted with H_2O and partitioned successively with EtOAc and *n*-BuOH. The EtOAc extract (600 g) was subjected to CC on silica gel using $CHCl_3$–EtOAc with increasing amounts of EtOAc, to give six frs. Fr. 1 was repeatedly chromatographed over silica gel to give **1** (11 mg), **2** (16 mg). Compound **3** (3 mg) was obtained from fr. 4 by CC on silica gel using $CHCl_3$–MeOH or ODS using MeOH–H_2O.

3.3. Plicatol A *1*

Colorless powder, IR ν_{max} cm^{-1}: 3592, 3267, 1614, 1539, 1334, UV λ_{max} nm: 260, 280, 320, ^1H- and ^{13}C- NMR spectral data: Table 1, MS *m*/*z* (rel. int.%): 300 [M$^+$] (100), 285 (41), 257 (79), 242 (45), 227 (22), HRMS: $C_{17}H_{16}O_5$. Found: 300.0997, calculated 300.0998.

3.4. Plicatol B **2**

Colorless powder, IR ν_{max} cm^{-1}: 3600, 3250, 1620, UV λ_{max} nm: 255, 285, 315, ^1H- and ^{13}C- NMR spectral data: Table 1, MS *m*/*z* (rel. int.%): 240 [M$^+$] (100), 225 (53), 212 (23), 197 (38), HRMS: $C_{15}H_{12}0_3$. Found: 240.0789, calculated 240.0786; diacetate: ^1H-NMR spectral data ($CDCl_3$): δ 2.34 (3H, *s*, OAc), 2.37 (3H, *s*, OAc), 3.87 (3H, *s*, OMe), 6.84 (1H, *d*, *J* = 2.1 Hz, H-3), 7.22 (1H, *d*, *J* = 2.1 Hz, H-1), 7.40 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-8), 7.57 (1H, *dd*, *J* = 7.7, 8.0 Hz, H-7), 7.69 (1H, *dd*, *J* = 7.7, 1.0 Hz, H-6), 7.52 (1H, *d*, *J* = 9.0 Hz, H-10), 7.62 (1H, *d*, *J* = 9.0 Hz, H-9), MS *m*/*z* (rel. int.%): 324 [M$^+$] (32), 282 (61), 240 (100), 225 (30).

3.5. Plicatol C **3**

Colorless powder, IR ν_{max} cm^{-1}: 3550, 3300, 1620, 1460, UV λ_{max} nm: 272, 304, ^1H- NMR spectral data: Table 1, MS *m*/*z* (rel. int.%): 258 [M$^+$] (100), 240 (53) HRMS: $C_{15}H_{14}O_4$. Found: 258.0896, calculated 258.0892.

Table 1
^1H- and ^{13}C- NMR spectral data for compounds **1**, **2** ($CDCl_3$) and **3** (CD_3OD)[a]

	1		**2**		**3**
	δ_H	δ_C	δ_H	δ_C	δ_H
1	7.39 *d* (2.6)	101.1	6.97 *d* (2.6)	107.5	6.52 *d* (2.6)
2		155.6		153.9	
3	6.79 *d* (2.6)	101.0	6.83 *d* (2.6)	101.9	6.56 *d* (2.6)
4		155.0		155.5	
4a		112.6		114.4	
4b		117.3		136.2	
5		153.9		154.5	
6	7.20 *dd* (7.7, 1.3)	116.2	7.23 *dd* (7.7, 1.3)	116.7	6.88 *dd* (8.1, 1.3)
7	7.51 *dd* (7.7, 8.1)[b]	127.3	7.48 *t* (7.7)	127.1	7.16 *dd* (7.7, 8.1)[b]
8	7.83 *dd* (8.1, 1.3)	113.8	7.41 *m*[c]	120.7	7.09 *br-dd* (7.7, 1.3)
8a		130.7		134.2	
9		145.0	7.61 *d* (9.0)	129.5	4.58 *dd* (10.0, 4.0)
10		142.4	7.41 *m*[c]	125.9	2.74 *dd* (10.0, 15.0, ax.), 2.96 *dd* (15.0, 4.0, eq.)
10a		133.1		118.8	
4-OCH_3	4.02	58.5	4.04	58.5	3.93
9-OCH_3	4.03	60.7			
10-OCH_3	3.99	60.6			
2-OH	5.73		5.68		
5-OH	9.43		9.64		

[a] Coupling constant (*J* in Hz) are given in parentheses.

[b] Triplet-like.

[c] Overlapped.

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