Constituents of *Ephemerantha fimbriata*. Isolation and Structure Elucidation of Two New Phenanthrenes, Fimbriol-A and Fimbriol-B, and a New Dihydrophenanthrene, Ephemeranthol-C[1)]

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Constituents of *Ephemerantha fimbriata* (BL.) P. F. HUNT *et* SUMMERH, which is used as a source plant of the Chinese crude drug "Shi-Hu", were examined and two new phenanthrenes, fimbriol-A (2) and fimbriol-B (8), a new dihydrophenanthrene, ephemeranthol-C (6), and dihydroconiferyl dihydro-*p*-coumarate (5) were isolated together with denbinobin (1), (+)-pinoresinol (3), (+)-syringaresinol (4), 3,4,5-trimethoxybenzoic acid (7), lusianthridin (9), and dihydro-*p*-coumaric acid (10). Structures of the new compounds were elucidated by the use of spectroscopic methods including two-dimensional NMR techniques.

Keywords *Ephemerantha fimbriata*; phenanthrene; dihydrophenanthrene; fimbriol-A; fimbriol-B; ephemeranthol-C

The Chinese crude drug "Shi-Hu (石斛)" is prepared from the dried stems of *Dendrobium nobile* and several other *Dendrobium* species (Orchidaceae), and is used as a tonic and an antipyretic.[2)] Some *Ephemerantha* species, such as *E. fimbriata*, *E. lonchophylla*, and *E. comata*, are also used as sources of "Shi-Hu."[3)] In a previous paper, we reported the isolation and the structures of five new compounds, ephemeranthol-A, ephemeranthol-B, ephemeranthoquinone, 3-*O*-methylgigantol, and ephemeranthoside, from the stems of *E. lonchophylla*.[1,4)] Recently, we have examined the constituents of *E. fimbriata* (BL.) P. F. HUNT *et* SUMMERH and isolated four new compounds, fimbriol-A (**2**), dihydroconiferyl dihydro-*p*-coumarate (**5**), ephemeranthol-C (**6**), and fimbriol-B (**8**), together with six known ones (**1**, **3**, **4**, **7**, **9** and **10**). This paper deals with the isolation and the structure elucidation of these compounds.

Stems of *Ephemerantha fimbriata* were cut into small pieces and extracted with ethanol at room temperature. The ether-soluble fraction of the ethanol extract was separated by silica gel column chromatography followed by preparative TLC to give ten compounds. Among them, five were identified as denbinobin (**1**),[1,5)] (+)-pinoresinol (**3**),[6)] (+)-syringaresinol (**4**),[6)] 3,4,5-trimethoxybenzoic acid (**7**), lusianthridin (**9**),[7)] and dihydro-*p*-coumaric acid (**10**) by analyses of spectral data and direct comparison with authentic samples.

Compound **5**, a colorless amorphous solid, showed the molecular ion peak at *m/z* 330 in the MS and its molecular formula was determined to be $C_{19}H_{22}O_5$ by high-resolution MS (HR-MS) measurement. Its UV and IR spectra closely resembled those of dihydro-*p*-coumaric acid (**10**) except for the appearanece of an absorption due to an ester group (ν1727 cm^{-1}) instead of a carboxylic acid group (ν3340, br and 1709 cm^{-1}) in the IR spectrum.

The ^{1}H-NMR spectrum of **5** showed signals due to two coupled methylenes (δ 2.59, 2.88, each 2H, t, J=8 Hz, 8-H_2, 7-H_2), three coupled methylenes (δ 1.89, 2H, quintet, J=8 Hz, 8′-H_2; 2.56, 2H, t, J=8 Hz, 7′-H_2; 4.08, 2H, t, J=8 Hz, 9′-H_2), a 1,4-disubstituted benzene (δ 6.75, 7.07, each 2H, d, J=8.5 Hz, 3,5-H_2, 2,6-H_2), and a 1,3,4-trisubstituted benzene (δ 6.64, dd, J=8.5, 1.5 Hz, 6′-H;

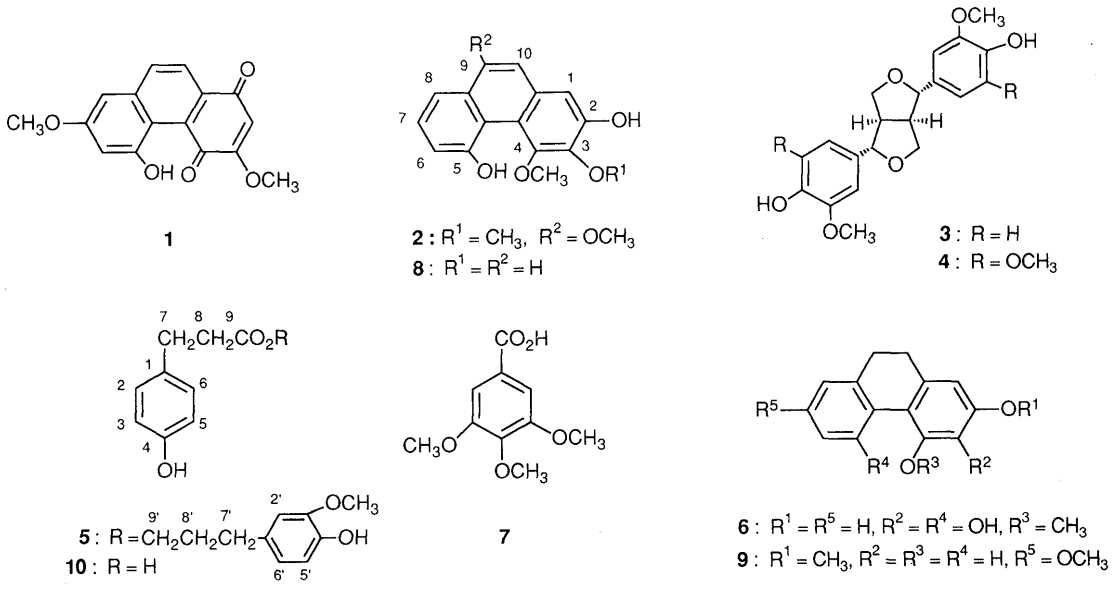

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Data for **2**, **6**, and **8** in $CDCl_3$

	2		**6**		**8**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	7.16 s	108.6 d	6.75 s	112.1 d	7.26 s	110.4 d
2		148.3 s		143.4 s		144.6 s
3		138.8 s		134.7 s		137.3 s
4		147.2 s		142.5 s		141.0 s
4a		113.0 s		117.6 s		115.7 s
4b		119.2 s		119.6 s		117.5 s
5		153.9 s		153.2 s		153.4 s
6	7.27 dd (8, 1.5)	117.2 d	6.96 dd (7.7, 1.3)	117.6 d	7.25 dd (7.5, 1.3)	115.6 d
7	7.52 t (8)	127.3 d	7.16 t (7.7)	128.4 d	7.50 t (7.5)	127.3 d
8	7.95 dd (8, 1.5)	114.1 d	6.86 dd (7.7, 1.3)	120.0 d	7.44 dd (7.5, 1.3)	120.8 d
8a		128.7 s		140.6 s		134.5 s
9		153.7 s	2.72 m	31.1 t	7.55 d (8.7)	126.8 d
10	6.75 s	101.7 d	2.63 m	30.1 t	7.47 d (8.7)	126.3 d
10a		131.6 s		133.3 s		128.1 s
3-OCH_3	4.14 s	62.3 q				
4-OCH_3	3.80 s	62.6 q	3.70 s	62.2 q	3.79 s	62.6 q
9-OCH_3	4.04 s	55.6 q				
2-OH	5.92 br s					
5-OH	10.1 s		8.24 s		10.2 s	

6.65, br s, 2′-H; 6.83, d, J=8.5 Hz, 5′-H) together with a methoxyl (δ 3.87, 3′-OC$\underline{H}_3$) and a hydroxyl group (δ 5.48, br s). In difference nuclear Overhauser effect (NOE) experiments, irradiation of the methylene protons at δ 2.56 (7′-H_2) and at δ 2.88 (7-H_2) showed NOE increases of the protons at δ 6.64 (6′-H) and 6.65 (2′-H) and at δ 7.07 (2,6-H_2), respectively, while irradiation of the methoxy protons (δ 3.87) showed an NOE increase of the proton at δ 6.65 (2′-H).

From these spectral data and the IR absorption of an ester carbonyl group, **5** was concluded to be dihydroconiferyl dihydro-*p*-coumarate. This conclusion was supported by the fragment ion at *m/z* 164 (base peak, $C_{10}H_{12}O_2$) formed by McLafferty fragmentation in the MS.

Fimbriol-A (**2**), a colorless amorphous solid, had the molecular formula $C_{17}H_{16}O_5$ as determined by HR-MS, and showed UV absorptions at 262, 274sh, 288sh, 302, 316, 329, 348, and 366 nm (log ε 4.45, 4.14, 3.88, 3.78, 3.76, 3.26, 3.43, 3.56), which are characteristic of phenanthrene derivatives.[8] The IR spectrum of **2** showed strong hydroxyl absorptions at 3529 and 3183 cm^{-1} and its ^{1}H-NMR spectrum revealed signals due to a 1,2,3-trisubstituted benzene ring (δ 7.27, dd, J=8, 1.5 Hz, 6-H; 7.52, t, J=8 Hz, 7-H; 7.95, dd, J=8, 1.5 Hz, 8-H) and two isolated benzene protons (δ 7.16, 1-H; 6.75, 10-H) along with three methoxyl (δ 3.80, 4.04, 4.14) and two hydroxyl protons (δ 5.92, 10.1). However, it showed no signals of *ortho*-coupled 9- and 10-protons of phenanthrene derivatives,[8] suggesting that **2** has at least a substituent at the C-9 or C-10 position.

Locations of the methoxyl and hydroxyl groups in **2** were determined by difference NOE experiments (Chart 2). Irradiation of the methoxyl protons at δ 4.04 (9-OC$\underline{H}_3$) gave an NOE enhancement of the proton at δ 6.75 (10-H) and irradiation of the latter proton increased the intensities of the former protons and the proton at δ 7.16 (1-H). In turn, irradiation of the proton at δ 7.16 (1-H) showed an NOE enhancement of the isolated proton at δ 6.75 (10-H). Thus, the methoxyl group at δ 4.04 was considered to be

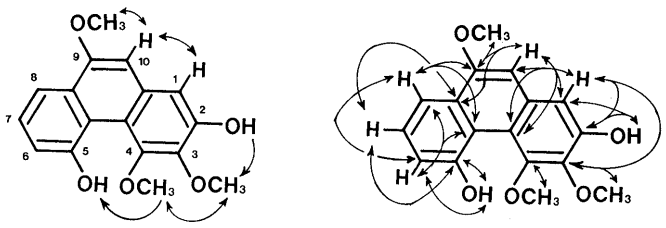

Chart 2. NOE's (Left) and Long-Range Correlations (Right) Observed in the Difference NOE and HMBC Spectra of **2**

located at the C-9 position and the two isolated benzene protons at the C-1 and C-10 positions. On the other hand, irradiation of the protons at δ 3.80 (4-OC$\underline{H}_3$), 4.14 (3-OC$\underline{H}_3$), and 5.92 (2-O$\underline{H}$) increased the intensities of the protons at δ 4.14 (3-OC$\underline{H}_3$) and 10.1 (5-O$\underline{H}$), at δ 3.80 (4-OC$\underline{H}_3$), and at δ 4.14 (3-OC$\underline{H}_3$), respectively. Therefore it was reasonable to assume that the two remaining methoxyl groups were located at the C-3 and C-4 positions and two hydroxyl groups at the C-2 and C-5 positions.

From these data, fimbriol-A was determined to be 2,5-dihydroxy-3,4,9-trimethoxyphenanthrene (**2**). This was supported by the long-range correlations observed in the ^{1}H-detected heteronuclear multiple-quantum multiple-bond correlation (HMBC) spectrum[9] (Chart 2). Fimbriol-A (**2**) is the second example of a ring-B oxygenated phenanthrene derivative obtained from a natural source.[10]

Ephemeranthol-C (**6**), a colorless amorphous solid, $C_{15}H_{14}O_4$, showed UV absorptions at 266, 271, 294sh, 307 nm (log ε 3.39, 3.38, 3.28, 3.34) and IR absorptions at 3450 (OH), 1604, 1523, and 1425 cm^{-1} (benzene ring). The ^{1}H-NMR spectrum of **6** showed signals assignable to 9-H_2 and 10-H_2 of dihydrophenanthrenes (δ 2.63, 2.72, each 2H, m),[1,8] a 1,2,3-trisubstituted benzene ring, an isolated benzene proton, and a methoxyl group (Table I). These spectral data suggested that **6** should be a dihydrophenanthrene having a methoxyl and three hydroxyl groups.

It should be noted that the ^{1}H-NMR spectrum showed

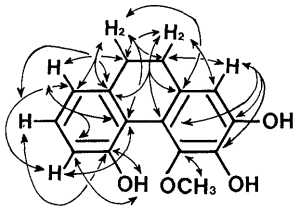

Chart 3. Long-Range Correlations Observed in the HMBC Spectrum of **6**

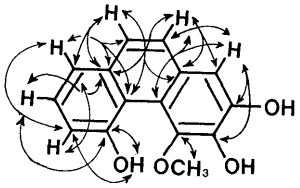

Chart 4. Long-Range Correlations Observed in the HMBC Spectrum of **8**

no signal at around δ 8 ppm, characteristic of the protons at the C-4 and C-5 positions of dihydrophenanthrenes,[1,8] indicating that both C-4 and C-5 are substituted. In difference NOE experiments, irradiation of the methylene protons at δ 2.63 (2H, m, 10-H_2) and at δ 2.72 (2H, m, 9-H_2) gave NOE enhancements of the isolated benzene proton at δ 6.75 (1H, s, 1-H) and the *ortho*-, *meta*-coupled benzene proton at δ 6.86 (1H, dd, J=7.7, 1.3 Hz, 8-H), respectively. Thus, **6** was supposed to be a 2,3,4,5-tetra-substituted dihydrophenanthrene.

The location of the methoxy group (δ 3.70) was determined to be at the C-4 position by careful analyses of the ^{1}H-detected heteronuclear multiple-quantum correlation (HMQC)[9b,11] and HMBC spectra (Chart 3).

From the data mentioned above, ephemeranthol-C was determined to be 2,3,5-trihydroxy-4-methoxy-9,10-dihydrophenanthrene (**6**).

Fimbriol-B (**8**) was obtained as an amorphous solid and its molecular formula was determined to be $C_{15}H_{12}O_4$ (*m/z* 256) by MS and HR-MS measurements. It showed characteristic UV absorptions of phenanthrene derivatives[8] and IR absorptions due to hydroxyl groups and aromatic rings (see Experimental). The ^{1}H-NMR spectrum of **8** showed signals due to a 1,2,3-trisubstituted benzene ring, an isolated benzene proton, and a methoxyl group along with signals due to a pair of *ortho*-coupled (J= 8.7 Hz) protons ascribable to 9-H and 10-H of a phenanthrene ring[8] (Table I). From these data, **8** was considered to be a phenanthrene derivative having a methoxyl and three hydroxyl groups.

Finally, fimbriol-B was determined to be 2,3,5-trihydroxy-4-methoxyphenanthrene (**8**) based on the analyses of the HMQC and HMBC spectra (Chart 4).

Experimental

UV spectra were obtained with a Shimadzu 202 UV spectrometer in EtOH solutions and IR spectra were taken with a Nicolet 5DX FT-IR spectrometer in $CHCl_3$ solutions. NMR spectra were measured with a JEOL GX-400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. MS and HR-MS were taken with a JEOL JMS D-300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. Column chromatography was done with Mallinkrodt silica gel and preparative TLC with Merck Kieselgel GF_{254} plates.

The crude drug Shi-Hu employed in this study was kindly provided by Tochimoto-tenkaido Co., Ltd. (collected in Vietnam and imported into Japan) and identified as *Ephemerantha fimbriata* (BL.) P. F. HUNT *et* SUMMERH by one of the authors (G.-J. Xu).

Extraction and Isolation Air-dried stems of *E. fimbriata* (159 g) were cut into small pieces and extracted with EtOH (1 l, 3 h, ×5) at room temperature. After concentration of the combined EtOH solution *in vacuo*, the residue was suspended in water and extracted successively with ether and EtOAc (each 300 ml × 6) to give an ether extract (4.60 g) and an EtOAc extract (1.30 g) along with an insoluble material (640 mg). The ether extract was chromatographed on a silica gel column with $CHCl_3$–MeOH (100:0, 99:1, 98:2, 96:4, 92:8), and eluates were separated into ten fractions according to TLC behavior.

Fraction 2 (86 mg) was subjected to preparative TLC with MeOH–$CHCl_3$ (1:99) to give denbinobin (**1**, 1.0 mg)[1,6] from the less polar zone and fimbriol-A (**2**, 1.1 mg) from the more polar zone.

Fraction 4 (61 mg) was separated by preparative TLC with $CHCl_3$–MeOH (98:2) to give (+)-pinoresinol (**3**, 1.0 mg),[7] (+)-syringaresinol (**4**, 1.0 mg),[7] dihydroconiferyl dihydro-*p*-coumarate (**5**, 2.3 mg), ephemeranthol-C (**6**, 2.5 mg), and 3,4,5-trimethoxybenzoic acid (**7**, 5.3 mg) in order of increasing polarity.

Fractions 5 (52 mg), 6 (40 mg), and 9 (55 mg) were also subjected to preparative TLC with MeOH–$CHCl_3$ (10:90, 1:99, 4:96, respectively). Fimbriol-B (**8**, 12.5 mg), lusianthridin (**9**, 4.2 mg),[10] and dihydro-*p*-coumaric acid (**10**, 5.9 mg) were obtained from frs. 5, 6, and 9, respectively.

Fimbriol-A (2) Colorless amorphous solid. UV λ_{max} nm (log ε): 262 (4.45), 274sh (4.14), 288sh (3.88), 302 (3.78), 316 (3.76), 329 (3.26), 348 (3.43), 366 (3.56). IR ν_{max} cm^{-1}: 3529, 3183 (br, OH), 1625, 1604, 1532, 1500, 1467, 1428 (benzene ring). ^{1}H- and ^{13}C-NMR: Table I. MS *m/z* (%): 300 (M^+, 100), 285 (65), 270 (5), 257 (13), 253 (11), 242 (40), 227 (10), 199 (6). HR-MS: Found 300.0997, Calcd for $C_{17}H_{16}O_5$ (M^+) 300.0997.

Dihydroconiferyl Dihydro-*p*-coumarate (5) Colorless amorphous solid. UV λ_{max} nm (log ε): 228 (3.82), 279 (3.65). IR ν_{max} cm^{-1}: 3596, 3549, 3315 (br, OH), 1727 (CO), 1605, 1515, 1465 (benzene ring). MS *m/z* (%): 330 (M^+, 75), 181 (5), 164 (100), 149 (16), 137 (26), 107 (13). HR-MS: Found 330.1463, Calcd for $C_{19}H_{22}O_5$ (M^+) 330.1467; Found 164.0837, Calcd for $C_{10}H_{12}O_2$ 164.0837.

Ephemeranthol-C (6) Colorless amorphous solid. UV λ_{max} nm (log ε): 266 (3.39), 271 (3.38), 294sh (3.28), 307 (3.34). IR ν_{max} cm^{-1}: 3450 (br, OH), 1604, 1523, 1425 (benzene ring). ^{1}H- and ^{13}C-NMR: Table I. MS *m/z* (%): 258 (M^+, 100), 243 (16), 225 (10), 197 (15), 169 (6). HR-MS: Found 258.0890, Calcd for $C_{15}H_{14}O_4$ (M^+) 258.0891.

Fimbriol-B (8) Colorless amorphous solid. UV λ_{max} nm (log ε): 262 (3.96), 303 (3.46), 315 (3.43), 330sh (3.16), 350 (3.17), 367 (3.19). IR ν_{max} cm^{-1}: 3546, 3256 (br, OH), 1645, 1618, 1592, 1544, 1508, 1470 (benzene ring). ^{1}H- and ^{13}C-NMR: Table I. MS *m/z* (%): 256 (M^+, 100), 241 (29), 223 (15), 213 (19), 196 (15), 138 (27), 128 (8). HR-MS: Found 256.0739, Calcd for $C_{15}H_{12}O_4$ (M^+) 256.0735.

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