Two New Compounds from *Dendrobium chrysotoxum*

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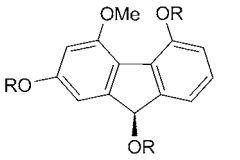
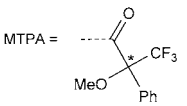
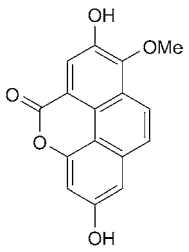
Two novel compounds, (9*R*)-4-methoxy-9*H*-fluorene-2,5,9-triol (**1a**) and 2,7-dihydroxy-8-methoxyphenanthro[4,5-*bcd*]pyran-5(5*H*)-one (**2**) were isolated from the stems of the orchid *Dendrobium chrysotoxum* Lindl. Their structures were established spectroscopically, by chemical derivatization, and by single-crystal X-ray diffraction analysis. The absolute configuration of **1a** was determined by *Mosher*'s method. Neither compound showed antitumoral activity.

Introduction. – The fresh or dried stems of *Dendrobium chrysotoxum* Lindl. are being used in both traditional Chinese and folk medicine for treatment of loss of appetite with nausea, fever in deficiency condition, after a severe disease, and impaired vision. In previous studies, we have reported the isolation of two new fluorenones, along with other known compounds (bibenzyls, phenanthrenes, fluorenones, *etc.*) from this plant, including erianin and chrysotobibenzyl, which exhibit significant antitumour activities against several tumour-cell lines such as A-549 and P388 [1–3]. Our continuing study on this plant now led to the isolation of a novel fluorenol, (9*R*)-4-methoxy-9*H*-fluorene-2,5,9-triol (**1a**), and a novel phenanthrene, 2,7-dihydroxy-8-methoxyphenanthro[4,5-*bcd*]pyran-5(5*H*)-one (**2**), obtained from EtOH extracts after repeated chromatographic purification (SiO_2; *Sephadex LH-20*) and recrystallization ($CHCl_3$/MeOH). Here, we report the isolation and structure elucidation of these two new compounds.



1a R = H
1b R = (*R*)-(+)-MTPA
1c R = (*S*)-(–)-MTPA





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Results and Discussion. – Compound **1a** was obtained as colorless needles (m.p. 209.9–210.0°). By EI-MS (*m/z* 244 (100, M^+)) and elemental analysis, the molecular formula $C_{14}H_{10}O_5$ was derived. The IR spectrum showed the presence of OH groups (3402 cm^{-1}) and C=C bonds (1607 cm^{-1}). The UV spectrum of **1a** showed absorption maxima at $\lambda = 286$, 278, 246, and 237 nm, similar to those of fluorenone derivatives [1]. The ^{13}C-NMR spectrum of **1a** (*Table 1*) indicated a total of 14 C-atoms, twelve of which resonating in the aromatic region, a MeO group, and an O-bearing tertiary C-atom. The ^{1}H-NMR spectrum (*Table 1*) showed five aromatic H-atoms (three on ring A, two on ring B), and two coupled resonances at δ_H 5.28 and 5.74 ppm ($2d$, $J = 7.7$ each), the latter disappearing and the former turning into a *s* in the presence of D_2O. The aromatic H-atoms on ring A showed an *ortho*-substitution pattern, while those on ring B displayed *meta*-substitution (δ_H 6.71 and 6.52 ($2d$, $J = 1.7$ each)). The above data, together with δ_C 73.6 and δ_H 5.28 ppm for H–C(9) were in accordance with a substituted fluoren-9-ol. The assignment was further confirmed by HMQC and HMBC experiments (see *Fig. 1*, *a* and *Table 1*). In the HMQC spectrum, the H-atom at 5.28 ppm (H–C(9)) correlated with the tertiary-C signal at δ_C 73.6 (C(9)). In the HMBC experiments, the H-atoms at δ_H 6.71 (d, $J = 1.7$, 1 H) and 7.10 ppm (d, $J = 7.2$, 1 H) correlated with δ_C 73.6 (C(9)), thus corresponding to H–C(1) and H–C(8), respectively. Accordingly, the signal at δ_H 6.52 ppm was assigned to H–C(3) (*meta* to H–C(1)), and the signals at δ_H 7.14 (t, $J = 7.2$, 1 H) and 7.10 (d, $J = 7.2$, 1 H) were ascribed to H–C(7) and H–C(6), respectively (*ortho*-related atoms). Both the signals at δ_H 4.03 (MeO) and 6.52 ppm (H–C(3)) correlated with that at δ_C 151.7 (C(4)). Therefore, the MeO group should be at C(4). Based on these considerations, the structure of **1a** was deduced as 4-methoxy-9*H*-fluorene-2,5,9-triol.

Table 1. *NMR Spectral Data of* **1a**[a]). Spectra were recorded at 400 (^{1}H) and 100 MHz (^{13}C) in (D_6)DMSO. Chemical shifts δ in ppm rel. to $SiMe_4$, coupling constants J in Hz.

	δ_H	δ_C	HMBC
H–C(1)	6.71 (d, $J = 1.7$)	106.2	C(2), C(3), C(9), C(4a)
C(2)	–	158.6	–
H–C(3)	6.52 (d, $J = 1.7$)	99.3	C(1), C(2), C(4), C(4a)
C(4)	–	151.6	–
C(5)	–	149.5	–
H–C(6)	6.73 (d, $J = 7.2$)	115.8	C(5), C(8), C(4b)
H–C(7)	7.14 (t, $J = 7.2$)	127.3	C(6)
H–C(8)	7.10 (d, $J = 7.2$)	116.1	C(6), C(7), C(9), C(4a), C(4b), C(8a)
H–C(9)	5.28 (d, $J = 7.7$)	73.6	C(1), C(8), C(4b), C(8a), C(9a)
C(9a)	–	149.7	–
C(4a)	–	116.9	–
C(4b)	–	123.5	–
C(8a)	–	147.6	–
MeO	4.03 (*s*)	56.6	C(4)
9-OH	5.74 (d, $J = 7.7$)	–	–
2,5-OH	9.11/9.75	–	–

[a]) Fluorene numbering (see *Fig. 1*, *a*).

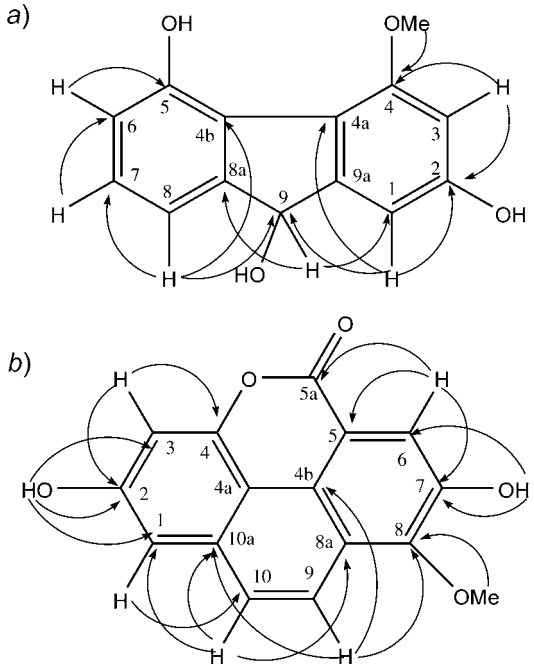

Fig. 1. *Key HMBC correlations in* a) **1a** *and* b) **2** (see also *Tables 1* and *3*)

Finally, the structure of **1a** was unequivocally confirmed by single-crystal X-ray diffraction analysis[1]), as shown in *Fig. 2* (see also *Exper. Part*).

To determine the absolute configuration at C(9), compound **1a** was converted to the two epimeric *Mosher* esters **1b** and **1c** by standard methodology [4–6]. Comparison of the relevant ^{1}H-NMR resonances (*Table 2*) established the (9*R*)-configuration; hence, compound **1a** corresponds to (9*R*)-4-methoxy-9*H*-fluorene-2,5,9-triol.

Compound **2** was obtained as yellow needles (m.p. 199.2–199.5° ($CHCl_3$/MeOH)). The molecular formula $C_{16}H_{10}O_5$ was established by HR-EI-MS (*m/z* 282.0547 (M^+, $C_{16}H_{10}O_5^+$; calc. 282.0528)). The IR spectrum showed bands for OH groups and a conjugated δ-lactone group. A total of 16 ^{13}C-NMR signals were found (*Table 3*), including resonances corresponding to 14 aromatic C-atoms (five CH, nine C_q), one MeO group, and one ester C=O group. In the ^{1}H-NMR spectrum of **2**, two OH groups and one MeO group were present according to resonances at δ_H 10.3 and 10.4 (2*s*, 2 × 1 H) and 4.08 (*s*, 3 H). The ^{1}H-NMR spectrum also displayed five aromatic H-atoms, appearing as a pair of *meta*-related doublets, a pair of *ortho*-related doublets, and a singlet. Hence, **2** was expected to incorporate a substituted phenanthrene nucleus and a conjugated δ-lactone.

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[1]) Crystallographic data (excluding structure factors) for the structure of **1a** have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-230680. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

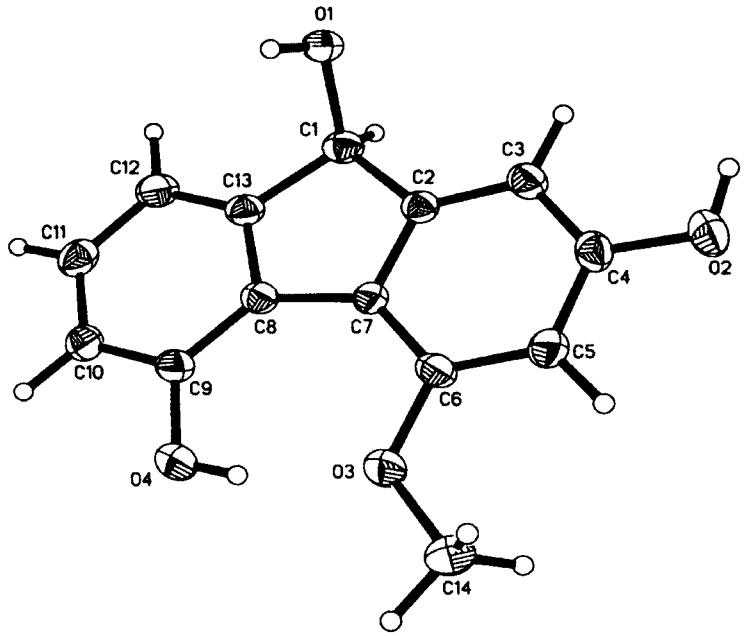

Fig. 2. *X-Ray single-crystal structure of* **1a**

Table 2. *600-MHz ^{1}H-NMR Spectral Data of* Mosher *Esters* **1b** *and* **1c** *in CD_3OD*

	1b	**1c**	$\Delta\delta$ (**1b**–**1c**)
H–C(1)	6.558	6.402	+0.156
H–C(3)	6.420	6.383	+0.037
H–C(6)	6.627	6.635	−0.008
H–C(7)	6.834	6.908	−0.074
H–C(8)	6.599	6.806	−0.207
H–C(9)	6.627	6.630	−0.003
MeO	3.858	3.844	+0.014

The *ortho*-related signals at δ_H 7.85 and 8.00 ppm (2*d*, $J = 9.2$ each, 2×1 H) were attributed to H–C(10) and H–C(9), respectively. The *meta*-related signals at 7.23 and 7.07 ppm (2*d*, $J = 2.0$ each, 2×1 H) were assigned to H–C(1) and H–C(3) accordingly. The singlet at 8.04 ppm (1 H) was, thus, due to H–C(6).

The above assignments were further confirmed by 2D-NMR analyses (see *Fig. 1,b* and *Table 3*). In the HMBC spectrum, the H-atom of C(6) correlated with both C(5) (δ_C 113.2) and the carbonyl C(5a)-atom (δ_C 160.2). The ^{1}H-NMR signal at δ_H 10.4 (OH) also correlated with C(6), *i.e.*, this OH group had to be located at C(7). Both the H–C(9) and the MeO H-atoms correlated with C(8), thus, the MeO group had to be at C(8). The second OH group was then placed at C(2), in accord with HMBC experiments. The above data supported that compound **2** corresponds to 2,7-dihydroxy-8-methoxyphenanthro[4,5-*bcd*]pyran-5(5*H*)-one.

Compounds **1a** and **2** were both inactive in a range of assays, including the A-549 and P388 antitumor assays. Compound **1a** is the first example of a fluorenol isolated

Table 3. *NMR Spectral Data of* **2**[a]). Spectra were recorded at 400 (^{1}H) and 100 MHz (^{13}C) in (D_6)DMSO. Chemical shifts δ in ppm rel. to $SiMe_4$, coupling constants J in Hz.

	δ_H	δ_C	HMBC
H–C(1)	7.23 (d, J = 2.0)	107.1	C(2), C(3), C(10)
C(2)	–	157.5	–
H–C(3)	7.07 (d, J = 2.0)	102.5	C(1), C(2), C(4)
C(4)	–	150.5	–
C(4a)	–	122.5[b])	–
C(4b)	–	122.6[b])	–
C(5)	–	113.2	–
H–C(6)	8.04 (s)	117.8	C(5), C(5a), C(7) or C(8), C(4b)
C(7)	–	147.9	–
C(8)	–	147.9	–
C(8a)	–	107.2	–
H–C(9)	8.00 (d, J = 9.2)	120.7	C(8), C(4b), C(10a)
H–C(10)	7.85 (d, J = 9.2)	126.3	C(10a), C(4a), C(8a) or C(1)
C(10a)	–	122.5	–
C(5a)	–	160.2	–
8-MeO	4.08 (s)	60.8	C(8)
2-OH	10.3 (s)	–	C(1), C(2), C(3)
7-OH	10.4 (s)	–	C(6), C(7)

[a]) Phenanthrene atom numbering (see *Fig. 1,b*). [b]) Signals may be interchanged.

from Orchidaceae. It is probably derived from the fluorenone dengibsin by reduction of the fluoren-9(9*H*)-one group.

Experimental Part

General. Column chromatography (CC): performed on silica gel (100–200 mesh) and *Sephadex LH-20* (*Pharmacia*). M.p.: *WRX-1-S* apparatus; uncorrected. UV Spectra: *Shimazu UV-2501* spectrophotometer, in MeOH; λ_{max} in nm. IR Spectra: *Nicolet Impact-410* apparatus, KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AFC-400* spectrometer, at 400 and 100 MHz, resp.; chemical shifts δ in ppm rel. to $SiMe_4$ (= 0 ppm) as internal standard. EI-MS (70 eV): on a *Hewlett-Packard 5989A* mass spectrometer; in *m/z* (rel. intensity in %).

Plant Material. Plants were collected at Xishuangbanna, Yunnan Province, China, in October 1999. The plants were identified as *Dendrobium chrysotoxum* Lindl. by Dr. *Xu Hong*. A voucher specimen was deposited at the Herbarium of China Pharmaceutical University, Nanjing, China.

Extraction and Isolation. Air-dried stems of *Dendrobium chrysotoxum* (12 kg) were powdered and extracted with $CHCl_3$ (3 ×). The residue was taken up in 95% EtOH, and the mixture was refluxed for 8 h. The solvent was evaporated, and the EtOH extract (400 g) was chromatographed (SiO_2; $CHCl_3$/MeOH 100:10) to yield a crude mixture of compounds (25 g), which was rechromatographed (SiO_2; $CHCl_3$/MeOH 100:5 → 100:20) to afford *Fractions I–VI*. *Fraction II* (which had been obtained at an elution gradient of $CHCl_3$/MeOH 100:6) was rechromatographed (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to yield **1a** (30 mg) and **2** (10 mg).

*(9*R*)-4-Methoxy-9*H*-fluorene-2,5,9-triol* (**1a**). Colorless needles. M.p. 209.9–210.0° ($CHCl_3$/MeOH). UV (MeOH): 286, 278, 246, 237. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 244 (100, M^+). Anal. calc. for $C_{14}H_{12}O_4$: C 68.85, H 4.95, O 26.20; found: C 68.76, H 5.00, O 26.24. X-Ray crystal structure: see *Fig. 2* and parameters given in the X-ray section below.

Preparation of Mosher *Esters* **1b** and **1c**. Compound **1a** (10 mg) in pyridine (0.5 ml) was separately treated with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic chloride[2]) (3 µl each), and the solns. were stirred

[2]) Systematic name: 3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (*Mosher*'s acid chloride).

at r.t. for 48 h. The solvent was evaporated, and the residues were purified chromatographically (*Sephadex LH-20*) to afford pure **1b** (2 mg) and **1c** (1.5 mg), respectively. ^{1}H-NMR: see *Table 2*.

2,7-Dihydroxy-8-methoxyphenanthro[4,5-bcd*]pyran-5(5*H*)-one* (**2**). Yellow needles. M.p. 199.2–199.5° ($CHCl_3$/MeOH). IR (KBr): 3406 (OH), 1708, 1632, 1592, 1417, 1343, 1277, 1156. UV (MeOH): 396.8, 379.6, 330.8, 269.8, 249.0. ^{1}H- and ^{13}C-NMR: see *Table 3*. EI-MS: 282 (100, M^+), 267 (95), 239 (45), 211 (8). HR-EI-MS: 282.0547 (M^+, $C_{16}H_{10}O_5^+$; calc.: 282.0528).

Crystallographic Data for **1a**. Data were collected at 293 K on a *Bruker SMART-CCD* area detector using graphite-monochromated MoK_α radiation. Colorless needles. Empirical formula: $C_{14}H_{12}O_4$. Formula weight: 244.24 g mol^{-1}. Crystal system: orthorhombic. Space group: $P2_12_12_1$. Unit-cell dimensions: $a = 5.0918(4)$, $b = 11.7965(11)$, $c = 18.9973(17)$ Å; $\alpha = \beta ? = \gamma = 90°$. $V = 1141.08(17)$ Å^3. $Z = 4$; $D_{calc} = 1.422$ Mg/m^3. $\mu = 0.105$ mm^{-1}. $F(000) = 512$.

This work was financially supported by the *National Science Foundation of China* (grant No. 30171144; *L.-S. X.*) and the *Science and Technology Development Fund of Shanghai* (grant No. 00XD14022; *Z.-T. W*). We thank Dr. *Xu Hong* and *Ding Xiaoyu*, Department of Pharmacognosy, China Pharmaceutical University, for providing the plant material.

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Received July 12, 2003