Leptosphaerone, a Metabolite with a Novel Skeleton from *Leptosphaeria* sp. IV403, an Endophytic Fungus in *Artemisia annua*

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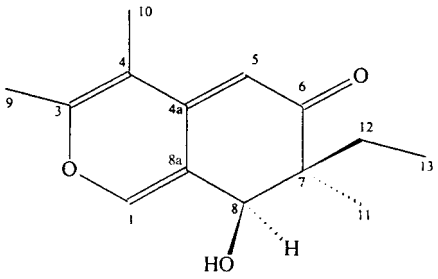
Leptosphaerone (**1**), a metabolite with a new C skeleton, was isolated from the AcOEt extract of the culture of *Leptosphaeria* sp., an endophytic fungus (strain number: IV403) found in *Artemisia annua*. The structure of **1** was elucidated on the basis of spectral analyses, including homo- and hetero-nuclear correlation NMR experiments (HMQC and HMBC), with its absolute configuration determined by CD and NOED studies.

1. Introduction. – Along with the accelerating advances of investigation of endophytes, it has been found that endophytes are a rich and reliable source of bioactive and/or chemically new compounds that may contain great medicinal or agricultural potential [1]. In our program devoted to the search for new bioactive metabolites from endophytes of *Artemisia* plants, we have found several antimicrobial compounds and have elucidated their structures [2][3]. As a part of this work, the isolated metabolite has a new C framework, named as *leptosphaerone* (**1**), which was obtained from *Leptosphaeria* sp., an endophytic fungus isolated from *Artemisia annua*. In this paper, the absolute configuration of **1** is reported.



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2. Results and Discussion. – Compound **1** was obtained as a yellowish gum with optical activity. Its molecular formula was determined to be $C_{14}H_{18}O_3$ on the basis of the high-resolution (HR) ESI mass spectrometry, showing an accurate protonated molecular ion at *m*/*z* 235.1329 ($[M+H]^+$), and NMR analysis. The UV spectra of **1**

exhibited absorption bands at 242 and 345 nm, characteristic of an α,β-unsaturated ketone. The IR spectra of **1** displayed absorption bands at 3419.3, 1715.0, 1668.4, and 1607.4 cm^{-1}, showing the existence of OH groups, and confirming an α,β-unsaturated ketone. A close inspection of the ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*) by distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence spectroscopy (HMQC) experiments revealed the presence of a conjugated ketone (C(6)), one tetrasubstituted C=C bond (C(3) and C(4)), two trisubstituted C=C bonds (C(1), C(8a), and C(4a), C(5)), one Et (C(12) and C(13)), three Me groups (C(9), C(10), and C(11)), and one sp^3-hybridized quaternary C-atom (C(7)). Moreover, ^{1}H- and ^{13}C-NMR signals (*Table*) suggested the presence of a tertiary OH group linked to C(8).

Table. *^{1}H-NMR* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data* ($CDCl_3$) *of Compound* **1**

Position	δ(C) (DEPT)	δ(H) (J in Hz)	HMBC
1	143.6 (CH)	7.32 (*s*)	H–C(8)
3	144.0 (C)		H–C(1), H–C(9), H–C(10)
4	111.4 (C)		H–C(5), H–C(10)
5	104.7 (CH)	5.36 (*s*)	
6	200.5 (C)		H–C(8), H–C(11), H–C(12)
7	49.8 (C)		H–C(8), H–C(11), H–C(12), H–C(13)
8	73.9 (CH)	4.46 (*s*)	H–C(11), H–C(12)
9	17.7 (Me)	2.22 (*s*)	
10	12.7 (Me)	1.87 (*s*)	
11	18.3 (Me)	1.16 (*s*)	H–C(8)
12	24.0 (CH_2)	1.61 (q, $J=10$)	H–C(8), H–C(11), H–C(13)
13	8.6 (Me)	0.86 (t, $J=10$)	
8a	119.3 (C)		H–C(1), H–C(5), H–C(8)
4a	153.9 (C)		H–C(1), H–C(9), H–C(10)

The analysis of heteronuclear multiple-bond coherence (HMBC) led to the elucidation of the C skeleton of **1**. The linkage of C(7) with C(8), C(11) and C(12) was established by HMBC correlations (*Table*) from C(7) to H–C(8), H–C(11), H–C(12), and H–C(13). The connection of C(6) and C(7) was verified by HMBC correlations (*Table*) between C(7) and H–C(5). The connectivity of C(1a) to C(8) was deduced from HMBC correlations (*Table*) for C(8a) to both H–C(5) and H–C(8). The O(2) anchored to C(1) and C(3) was revealed by both the chemical-shift value of H–C(1) and the HMBC correlation (*Table*) for C(3) to H–C(1) [4]. The evidence summarized above led to the planar structure of **1** as 7-ethyl-7,8-dihydro-8-hydroxy-3,4,7-trimethyl-6*H*-[2]benzopyran-6-one. To our knowledge, the C skeleton of **1** has not been reported until now.

The absolute configuration of **1** was established by a combination of the circular dichroism (CD) spectra and nuclear *Overhauser* effect difference (NOED) spectroscopy. The CD spectra of **1** showed a positive *Cotton* effect at 310 nm ($\Delta\varepsilon = +3.01$) due to the $n \rightarrow \pi^*$ transition of conjugated kentone (C(6)), which allowed the assignment of the absolute configuration as (7*S*) for **1** [5]. The observation of NOED (*Fig. 1*) between H–C(8) and H–C(11) suggested that H–C(8) is oriented *cis* to C(11), implying the establishment of absolute configuration as (8*R*) for **1**.

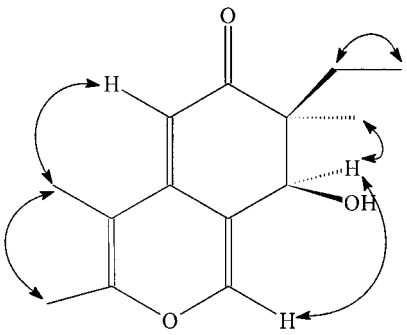

Fig. 1. *NOED Correlations for compound* **1**

Based on the above information, a computer-generated plot for the 3D structure of **1** (*Fig. 2*) was obtained with the molecular-modeling program CS CHEM 3D V5.0 by MM2 force-field calculations for energy minimization. The calculated distances between H–C(8)/H_α–C(11) (2.483 Å), H–C(8)/H–C(1) (3.096 Å), H–C(5)/H_α–C(10) (2.882 Å), H_β–C(10)/H_α–C(9) (2.149 Å), and H_β–C(12)/H_β–C(13) (2.471 Å) are all less than 4.00 Å, which is consistent with the well-defined NOEs observed for each of these proton pairs. Thus, the structure of **1** is (7*S*,8*R*)-7-ethyl-7,8-dihydro-8-hydroxy-3,4,7-trimethyl-6*H*-[2]benzopyran-6-one.

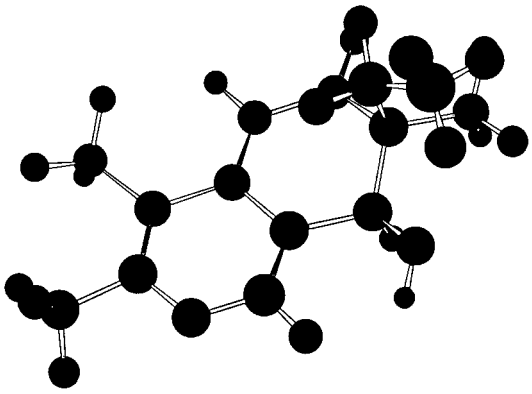

Fig. 2. *The 3D structure of compound* **1**

Experimental Part

General. All chemicals used in the study were of anal. grade. Optical rotations: *JASCO DIP-181* spectrometer. UV Spectra: *Hitachi U-3000* spectrophotometer. CD Spectra: *J-20C* automatic spectropolarimeter. IR Spectra: *Nexus 870* FT-IR; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR, and DEPT, HMQC, HMBC, and NOED Spectra: *Bruker DRX-500* spectrometer; at 500 and 125 MHz resp.; δ in ppm rel. to Me_4Si as an internal standard, *J* in Hz. HR-ESI-MS (positive-ion mode): *VG-ZAB-HS* mass spectrometer.

Material. Leptosphaeria sp., strain number IV403, is an endophytic fungus isolated from fresh stems of an apparently healthy *Artemisia annua* collected in May, 1997, in the suburb of Nanjing, China [6]. The culture of *Leptosphaeria* sp. was obtained according to the process reported in [3].

Extraction and Isolation. The culture filtrate (total volume 100 l) and mycelium were extracted with AcOEt. Evaporation of solvent *in vacuo* gave a residue (20 g), which was then subjected to column chromatography (CC) on silica gel (500 g, 200–300 mesh), eluting with petroleum ether/acetone (1:0–0:1) to give five fractions (*Fr. 1:* 10.0 g; *Fr. 2:* 3.5 g; *Fr. 3:* 2.2 g; *Fr. 4:* 4.0 g; *Fr. 5:* 1.5 g). *Fr. 3* was subjected to silica-gel chromatography (100 g, 200–300 mesh) with $CHCl_3$/MeOH (20:1–10:1). Three fractions were obtained (*Fr. 4.1:* 0.9 g; *Fr. 4.2:* 0.5 g; *Fr. 4.3:* 0.6 g). Further CC separation of *Fr. 4.1* over silica gel with $CHCl_3$/MeOH 10:1 (0.4 l), followed by gel filtration repeatedly over *Sephadex LH-20* with $CHCl_3$/MeOH 1:1, gave **1** (8 mg).

Leptosphaerone (=*(7S,8*R*)-7-Ethyl-7,8-dihydro-8-hydroxy-3,4,7-trimethyl-6*H*-[2]benzopyran-6-one*): yellowish gum. $[\alpha]_D^{25} = +225.5$ ($c = 0.15$, $CHCl_3$). UV ($CHCl_3$): λ_{max} 345, 242. IR: 3419.3, 2961.6, 2923.0, 1715.0, 1668.4, 1607.0, 1538.4, 1460.3, 1381.4, 1240.6, 1158.4, 1078.6, 758.5. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR ($CHCl_3$): *Table*. HR-ESI-MS: 235.1321 ($[M+H]^+$, $C_{14}H_{19}O_3^+$; calc. 235.1329).

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REFERENCES

[1] R. X. Tan, W. X. Zou, *Nat. Prod. Rep*. **2001**, *18*, 448.
[2] W. X. Zou, J. C. Meng, H. Lu, G. X. Chen, G. X. Shi, T. Y. Zhang, R. X. Tan, *J. Nat. Prod*. **2000**, *63*, 1529.
[3] H. Lu, W. X. Zou, J. C. Meng, J. Hu, R. X. Tan, *Plant Sci*. **2000**, *151*, 67.
[4] C. A. Boros, F. R. Stermitz, *J. Nat. Prod*. **1990**, *53*, 1060.
[5] A. Yajima, K. Mori, *Tetrahedron Lett*. **2000**, *41*, 351.
[6] C. H. Liu, W. X. Zou, H. Lu, R. X. Tan, *J. Biotechnol.* **2001**, *88*, 277.

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