ЖУРНАЛ СТРУКТУРНОЙ ХИМИИ

2012. Том 53, № 4 *Июль – август* *С. 796 – 801*

UDC 548.737:547.12

STRUCTURE AND STEREOCHEMICAL ASSIGNMENT OF SPHEROPSIDONE, A PHYTOTOXIN FROM *DIPLODIA CUPRESSI*

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Received January, 24, 2011

Sphaeropsidone is a phytotoxin produced in very large amount from *Diplodia cupressi*. It crystallizes in the monoclinic $P2_1$ space group with two molecules in the asymmetric unit. Cell parameters are: $a = 4.1280(6)$, $b = 13.161(1)$, $c = 13.333(3)$ Å, $\beta = 90.14(1)°$, $Z = 4$, $D_{cal} = 1.432$ Mg/m^3 at 173 K. The final refinement converged to $R_1 = 0.0413$, $wR_2 = 0.0730$ for 1684 observed reflections. In sphaeropsidone, the hydroxyl group and oxirane oxygen atoms are mutually *cis* positioned. The absolute stereochemistry at the three chiral centres turns out to be 1*S*,5*R*,6*S*. The stereochemical assignment of spaeropsidone is a fundamental basis for the assignment of the absolute configuration of some of its derivatives used in structure-activity relationship studies. No intramolecular hydrogen bonds are found. In the crystal packing, the hydroxyl and carbonyl oxygen atoms are involved in head-to-tail intermolecular hydrogen bonds to form infinite linear chains of molecules running along *c*. The linear chains are arranged into layers of molecules stacked along *a*.

K e y w o r d s: spheropsidone, phytotoxin, *Diplodia cupressi*, X-ray structure, derivative stereochemistry.

INTRODUCTION

The fungi associated with Cypress canker [1—9] produce several phytotoxic metabolites having different chemical nature, such as butenolides, bi- and tri-cyclic sesquiterpenes, macrolides, isobenzofuranones, and pimarane diterpenes (named sphaeropsidins A—C and A—F) [10—12]. Sphaeropsidone (5-hydroxy-4-methoxy-7-oxabicyclo[4.1.0]hept-3-en-2-one) and its 5-epimer (*epi*-sphearopsidone) are two phytotoxic dimedone methyl ethers that were first isolated, but only in a limited amount, in the culture filtrate of *Diplodia cupressi*, the casual agent of a canker disease of cypress in the Mediterranean area [13] (**1** and **2**, Fig. 1).

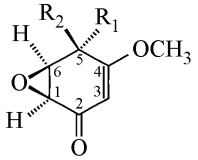

Fig. 1. Molecular scheme of sphaeropsidone (**1**) and *epi*-sphaeropsidone (**2**)

Sphaeropsidone (**1**) and *epi*-sphaeropsidone (**2**) appeared to be closely related to the other well known fungal metabolites [14—16] and showed a lower antifungal activity than sphaeropsidins [13], whose relationship between the structure and biological activity was extensively studied [17]. These results suggested a potential of the sphaeropsidones metabolites as natural fungicides against the cypress pathogen *Seiridium* sp. and stimulated further studies of the structure-activity relationships of these toxins [18]. These studies need a certain assignment of the relative and absolute stereochemistry to sphaeropsidones. The X-ray structural analysis reported here

could contribute to confirm the structure and relative stereochemistry previously assigned as 1*S*,5*R*,6*S* to (-)-spheropsidone and 1*S*,5*S*,6*S* to (-)-epispheropsidone (Fig. 1) by application of the time-dependent density functional theory (TDDFT) calculation of the optical rotation [19].

EXPERIMENTAL

Production, Extraction and Purification of Sphaeropsidone 1. The strain of *D. cupressi* used in this study was purchased from Centraalbureau voor Schimmelcultures of Baarn (Netherland), Strain 261.85 CBS. The fungus was grown in 2-liter Erlenmayer flasks containing 400 ml of modified Czapek medium supplemented with 2 % corn meal (pH 5.7). Each flask was seeded with 5 ml of a mycelia suspension, and then incubated at 25°C for 4 weeks in darkness. The fungal culture filtrates were worked up as previously reported [13]. The organic extract, obtained as a brown-red oil (9.2 g), having high phytotoxic activity, was chromatographed by silica gel column chromatography eluted with $CHCl_3$-*iso*-PrOH (19:1, v/v) affording 9 groups of homogeneous fractions. The residue (3.6 g) of fractions 4-7 were combined and further purified by silica gel column chromatography eluted with $CHCl_3$-*iso*-PrOH (9:1, v/v), yielding 6 groups of homogeneous fractions. The residue of fraction 3 was crystallized from EtOAc-*n*-hexane (1:5, v/v) yielding sphaeropsidone (**1**, R_f 0.40, 2.3 g, 153.3 mg/l) as white needles. This was re-crystallized from chloroform-benzene (1:1, v/v) and gave white crystals that were suitable for single crystal X-ray analysis.

X-ray Diffraction Study of Sphaeropsidone 1. White block shaped single crystals of sphaeropsidone **1** were obtained by slow evaporation of a chloroform-benzene solution at ambient temperature. X-ray data collection was performed at 173 K on a Bruker-Nonius Kappa CCD diffractometer equipped with graphite monochromated MoK_α radiation (λ = 0.71073 Å, φ scans and ω scans to fill the asymmetric unit). Cell parameters were obtained from a least-squares fit of the θ angles of 109 reflections in the range $5.235 \le \theta \le 20.272°$. A semiempirical absorption correction (multi-scan, SADABS) was applied. The structure was solved by direct methods and anisotropically refined by the full matrix least-squares method on F^2 against all independent measured reflections (SHELXS97 and SHELXL97 programs [20]). The positions of hydroxyl H atoms were determined from difference Fourier maps and refined according to a riding model; all other H atoms were placed in calculated positions and allowed to ride on carrier atoms (C—H in the 0.95—1.00 Å range; $U_{iso}(H) = 1.2U_{eq}$ (C methine or aromatic), $U_{iso}(H) = 1.5U_{eq}$ (C methyl) of the attached atom). Crystals resulted twinned by 180° rotation around the *a*-axis and a TWIN 1 0 0 0 –1 0 0 0 –1 instruction of the SHELXL97 program was used in the refinement. In the absence of any significant anomalous scattering, the absolute configuration was set by reference to the previous studies [19]. The final refinement converged to $R1 = 0.0413$, $wR2 = 0.0730$ for 1684 observed reflections having $I > 2\sigma(I)$. The minimum and maximum residual electronic density was –0.168 e/Å^3 and 0.154 e/Å^3. Crystal data and structure refinement details are reported in Table 1.

Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 794356. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033.

RESULTS AND DISCUSSION

(-)-Spheropsidone and (-)-episphaeropsidone (**1** and **2**, Fig. 1) were isolated for the first time, but only in a limited amount, from the culture filtrates of *D. cupressi* isolated from cortical tissues of infected cypress (*Cupressus sempervirens* L.) tree collected in Morocco and Italy [13].

In the present study, the strain used produces sphaeropsidones **1** and **2** in a very larger amount (153.3 and 48.3 mg/l) in respect to the yield (15.7 and 23.3 mg/l) obtained previously [13]. The availability of such a large amount of these bioactive cyclohexene oxides allowed an easy crystallization of sphaeropsidone **1** for the X-ray analysis here reported, and also allowed us to carry out extensive studies on their structure-activity relationships and their mode of action, which are partly in progress and partly already planned.

Table 1

Crystal data and structure refinement details for **1**

Chemical formula	$C_7H_8O_4$
Formula weight	156.13
Temperature, K	173(2)
Radiation, wavelength, Å	MoK_α, 0.71073
Crystal system	Monoclinic
Space group	$P2_1$
a, *b*, *c*, Å; β, deg.	4.1280(6), 13.161(2), 13.333(3); 90.14(1)
V, Å^3	724.4(2)
Z, D_{calc}, Mg/m^3	4, 1.432
Absorption coefficient, mm^{-1}	0.119
Theta range, deg.	3.06—27.48
Reflections collected / unique	4603 / 1684 [*R*(int) = 0.0444]
Data / restraints / parameters	1684 / 1 / 202
Final *R* indices, [$I > 2\sigma(I)$]	*R*1 = 0.0413, *wR*2 = 0.0730
R indices (all data)	*R*1 = 0.0899, *wR*2 = 0.0850
Largest diff.peack and hole, e/Å^3	0.154 and –0.168
CCDC deposition number	794356

An Ortep [21] view of sphaeropsidone **1** is shown in Fig. 2; selected bond lengths, bond angles, and torsion angles are reported in Table 2.

Compound **1** crystallizes in the $P2_1$ space group with two independent molecules in the asymmetric unit (A and B) with slightly different conformations, which are joined by the intermolecular OH···O=C hydrogen bond (Fig. 2).

Bond lengths and angles in **1** are in the normal range and in agreement with those of similar compounds [22—24]. In the six-membered ring, the geometry around C2, C3, and C4 confirms the presence of the cycloexene double bond and of the ketone group (C3—C4 = 1.337(7) Å, 1.334(7) Å, C2—O1 = 1.223(6) Å, 1.220(7) Å for A and B). The shortness of the C1—C6 bond length reflects the strain due to the epoxide ring (C1—C6 = 1.463(7) Å, 1.453(7) Å for A and B).

As already found [15, 25], the cycloexene ring adopts a very flat *boat* conformation with C2 and C5 atoms flipping away from the oxirane oxygen atom. In fact, the maximum deviation from the

Table 2

Selected bond lengths (Å), *bond angles* (deg.), *and torsion angles* (deg.) *with* e.s.d.′s *in parentheses*

	A	B		A	B
O1—C2	1.223(6)	1.220(7)	C1—C6	1.463(7)	1.453(7)
O2—C1	1.430(6)	1.440(7)	C2—C3	1.434(8)	1.428(8)
O2—C6	1.425(6)	1.411(6)	C3—C4	1.337(7)	1.334(7)
O3—C5	1.425(6)	1.405(6)	C4—C5	1.495(6)	1.497(7)
C1—C2	1.470(9)	1.477(9)	C5—C6	1.487(7)	1.484(6)
C2—C1—C6	119.8(5)	118.7(5)	C4—C5—C6	114.0(4)	111.9(4)
C1—C2—C3	118.1(5)	117.6(5)	C4—C5—O3	110.8(4)	112.6(4)
C2—C3—C4	121.9(5)	120.7(5)	C1—C6—C5	120.0(4)	120.3(4)
C6—C1—C2—C3	8.5(8)	17.2(8)	C3—C4—C5—C6	13.4(7)	22.7(8)
C1—C2—C3—C4	–8.3(8)	–17.9(8)	C4—C5—C6—C1	–12.3(7)	–21.0(7)

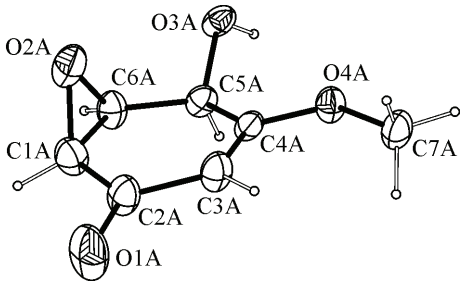

Fig. 2. ORTEP view of **1** showing one of the independent molecules (A).
Thermal ellipsoids are drawn at a 30 % probability level

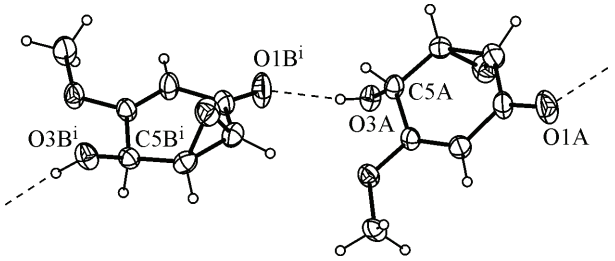

Fig. 3. Intermolecular OH···O=C head-to-tail hydrogen bonding pattern between the two independent molecules A and B.
The roughly eclipsed conformation of the H—O3—C5—H fragment is shown (i = –*x*+2, 1/2+*y*–1, –*z*–1)

C1/C3/C4/C6 least square plane is 0.002(3) Å for A and 0.003(3) Å for B; C2 and C5 distances from the least square plane are 0.094(9) Å and 0.152(8) Å for A (0.192(9) Å and 0.261(9) Å for B).

The methoxy group is quite in plane with the cycloexenone ring with the methyl group pointing towards the vinyl hydrogen atom. The hydroxyl group and the epoxide ring are mutually *cis* positioned and the absolute configuration at C1,C5,C6 was assigned as 1*S*,5*R*,6*S* based on the previous theoretical study [19]. In the same study [19], a high energy barrier was found to prevent the free rotation of the OH group and in the most stable conformer, the oxirane O atom was involved as acceptor in the intramolecular OH···O hydrogen bond. As a consequence, the *anti* arrangement of hydrogen atoms in the H—O—C—H fragment was observed. At variance with these results, no intramolecular hydrogen bonds are found in the structural study of **1** reported here (Fig. 3).

Only intermolecular interactions are present in the crystal. The OH group is, in fact, involved in a strong intermolecular hydrogen bond with the carbonyl oxygen atom of an adjacent molecule, while the oxirane O atom is involved only in weak CH···O intermolecular interactions (Table 3). As a consequence (Fig. 3), a roughly eclipsed conformation of the H—O3—C5—H fragment is observed.

In the crystal packing, the molecules are head-to-tail joined through strong intermolecular OH···O=C hydrogen bonds. In this way, infinite linear chains of molecules having the $C_2^2(14)$ graph set symbol [26] and running along the [0 0 1] direction are formed (Fig. 4).

Table 3

Hydrogen bonding geometry (e.s.d.′s *in parentheses*)

D—H···A	D—H (Å)	H···A (Å)	D···A (Å)	D—H···A (deg.)
O3A—H···O1B^{a}	0.79	1.97	2.718(6)	157.8
C6A—H···O3A^{b}	1.00	2.89	3.437(6)	115.5
C1A—H···O2A^{b}	1.00	2.65	3.237(6)	117.4
C5A—H···O3A^{b}	1.00	2.65	3.443(6)	136.3
C5B—H···O3B^{b}	1.00	2.47	3.445(6)	163.9
C6A—H···O2A^{b}	1.00	2.73	3.281(6)	114.7
C1A—H···O4B^{c}	1.00	2.48	3.278(6)	136.7
C1B—H···O4A^{d}	1.00	2.45	3.421(6)	162.3
O3B—H···O1A^{e}	0.95	1.85	2.777(5)	164.3

Symmetry code: ^{a} –*x*+2, *y*–1/2, –*z*–1; ^{b} *x*+1, *y*, *z*; ^{c} –*x*+2, *y*–1/2, –*z*; ^{d} –*x*+2, *y*+1/2, –*z*–1; ^{e} –*x*+2, *y*+1/2, –*z*.

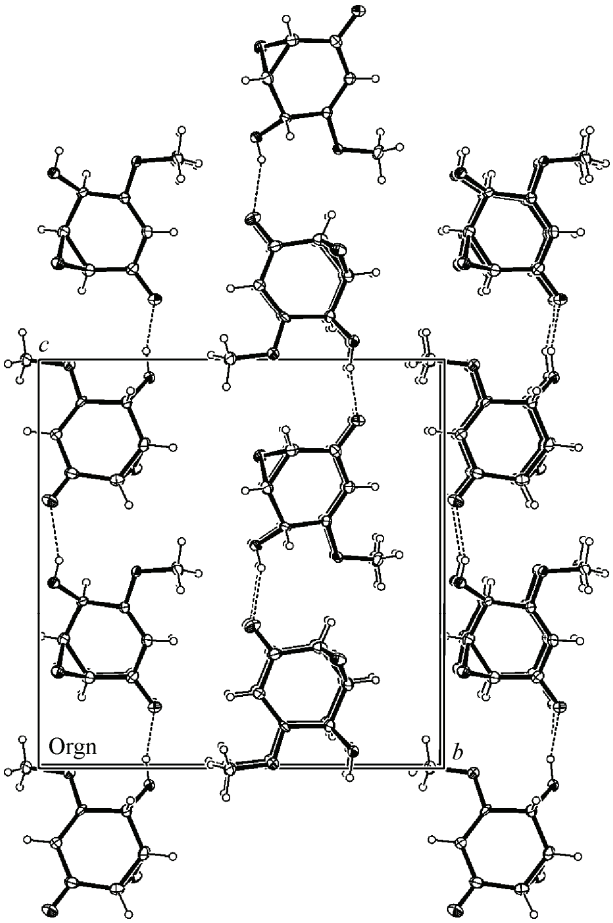

Fig. 4. Crystal packing viewed along the *a*-axis showing the hydrogen bonded chains of molecules. The intermolecular OH···O hydrogen bonds are drawn as dashed lines

The infinite chains of molecules are arranged into layers that roughly lie in the (0 1 1) plane. In this way, a stacking of layers of molecules in the [1 0 0] direction is obtained. Only weak CH···O intermolecular interactions are found between the adjacent layers (Fig. 5).

CONCLUSIONS

Although the conformation of the compounds in solution could be affected by the interaction with the solvent, the results reported here to confirm the relative and absolute configuration assigned to both spheropsidone **1** and its 5-epimer *epi*-sphaeropsidone **2**, are a fundamental basis in the structure-activity relationship studies carried out on these two dimedone methyl ethers [18]. In such a study, eight derivatives obtained by chemical modifications and two natural analogues were assayed for phytotoxic and antifungal activities, and a structure-activity relationship was examined. An important aspect was the assignment of the absolute configuration to new chiral carbons present in some of these derivatives, which probably affected their activity. The assignment was essentially based on the couplings measured in their ^{1}H NMR spectra, but starting from the relative and absolute stereochemistry assigned to sphaeropsidone. The results obtained provide insights into the structure-activity relationships within these compounds. It was found that the hydroxyl group at C-5, the absolute configuration of chiral carbon at position 5, the epoxy group and the carbonyl group at C-2 appear to be structural features important in conferring biological activity. The conversion of sphaeropsidone into

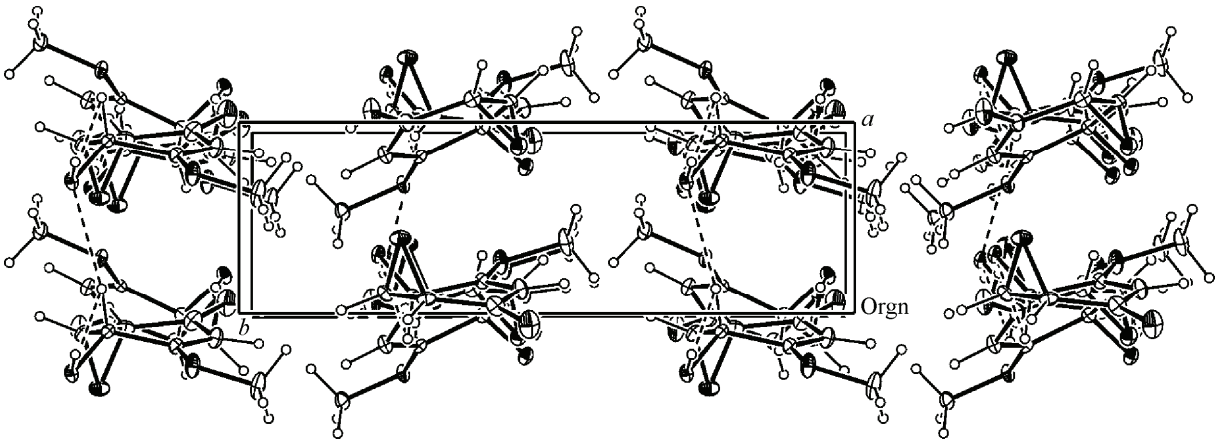

Fig. 5. Crystal packing viewed along the *c*-axis showing the stacking of layers of molecules along the *a*-axis. Dashed lines indicate the intermolecular CH···O interactions

the corresponding 1,4-dione derivative led to a compound showing greater antifungal activity than its precursor. This finding could be useful in devising new natural fungicides for practical application in agriculture [18].

Acknowledgments. The authors thank the Italian Ministry of University and Research (MIUR) for financial support. The research was also supported by a grant from Regione Campania L.R. 5/02. Contribution DISSPAPA 266. Thanks are also due to Centro Interdipartimentale di Metodologie Chimico Fisiche (CIMCF) of University of Naples ″Federico II″ for X-ray facilities.

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