Pergamon

Tetrahedron Letters, Vol. 35, No. 7, pp. 1043-1046, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0001-E

CLADOSPIRONE BISEPOXIDE - A NOVEL FUNGAL METABOLITE STRUCTURE DETERMINATION

Regina Thiergardt, Paul Hug†, Grety Rihs†, Heinrich H. Peter

Pharmaceutical Research Department, Microbial Chemistry and Central Function Research†
Ciba Geigy Ltd., 4002 Basle, Switzerland

Abstract: The structures of the new biologically active metabolite cladospirone bisepoxide **1** and its derivative 4-oxo-cladospirone bisepoxide **2** have been elucidated by manifold spectroscopic and X-ray crystallographic analyses.

Cladospirone bisepoxide **1** is a novel metabolite isolated from cultures of a saprophytic fungus [1]. Based on the morphological characteristics the producing strain was identified as a representative of the species *Cladosporium chlorocephalum* Fresen (Mason and Ellis) [2]. The fermentation and isolation procedures for the preparation of the metabolite and its biological activity will be reported separately [3].

This paper deals with the structure elucidation of cladospirone bisepoxide **1** and its derivative **2** on the basis of spectroscopic evidence and X-ray analysis.

1 was purified by silica gel column chromatography (ethyl acetate/5% isopropanol) to give colourless crystals (ethyl acetate). The mass spectrum of **1** showed a molecular ion peak [M^+] at *m/z* 366 (100%) requiring a molecular formula of $C_{20}H_{14}O_7$. Acetylation of **1** with acetic anhydride/pyridine gave a bisacetate, FAB-MS *m/z* 452 [M^+]. Absorption bands in the IR spectrum (KBr) of **1** underlined the presence of an alcohol (3460/3400 cm^{-1}), an α,β unsaturated ketone (1690/1680), a naphthalene ring (1640/1610/1585/1415/1380) and an aryl-O fragment (1275). These findings were emphasised by the characteristic UV spectrum [λ_{max} (ε) = 328 nm (5460), 313 (6600), 299 (8770), 226 (68110)].

A 1',8'-di-O-substituted naphthalene substructure was established by the appearance of two ABC systems at 7.59/7.60 (4'-, 5'-H), 7.47/7.51 (3'-, 6'-H) and 6.98/7.06 ppm (2'-, 7'-H) in the ^{1}H NMR spectrum of **1** (DMSO-d_6). In the low-field region, two protons at 5.94 and 5.61 ppm, interchangeable with deuterium oxide, could be assigned to two aliphatic hydroxyl groups (4-OH, 8-OH). Two signals at 5.84 (dd, 2-H; $J_{2,3}$ = 10.5, $J_{2,4}$ = 1.0 Hz) and at 6.72 ppm (dd, 3-H; $J_{2,3}$ = 10.5; $J_{3,4}$ = 4.9 Hz) were classified as an enone unit. An additional coupling between 2-H and 4-H (J = 1.0 Hz) could be revealed by 2D COSY experiments. It is obvious that only limited structural information was obtained from the chemical shifts and the interproton couplings. In addition, long range couplings discovered by recording of coupled ^{13}C NMR spectra with selective irradiation of appropriate proton frequencies were essential for the determination of the structure. Important was the observation that irradiation of 3-H affected the signal for C-10, as this confirmed a link between C-4 and C-10. The most significant multiple bond correlations detected with the aid of low power selective decoupling and heteronuclear correlation experiments are shown in figure 1 by arrows.

C	H	^3J (Hz)
C-1	3-H	11.6
C-2	4-H	4.9
C-4	2-H	9.0
C-10	3-H	4.7
C-10	8-H	4.7
C-10'	3'-H	8.9
C-10'	6'-H	8.9

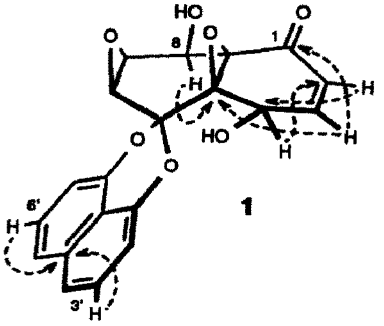
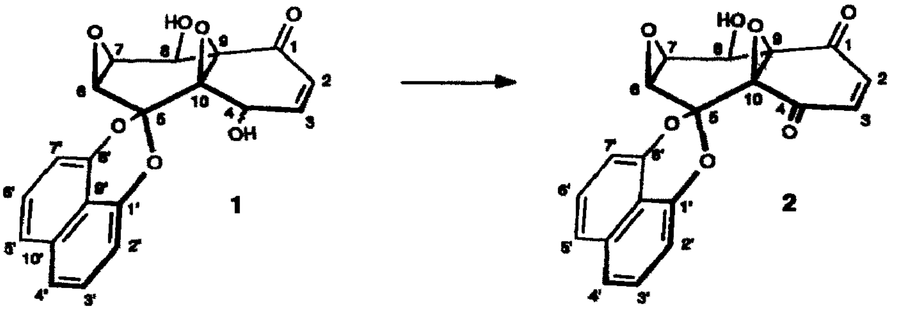


Figure 1: ^{13}C, ^1H Long range couplings for 1

In order to prove the suggested structure of cladospirone bisepoxide and to determine its reactivity, **1** was transformed in the endione **2** with MnO_2 in methylene chloride (25°C, 30 min). The physical and chemical properties of the compounds **1** and **2** are given in table 1, the NMR data in table 2.



	1	2
Appearance	colourless crystals	pale yellow crystals
MP (°C)	> 160°C (dec.)	>200°C (dec.)
Element. Analysis **Observed** **Calculated**	$C_{20}H_{14}O_7 \cdot 0.25\ H_2O$ C 64.54 H 4.09 C 64.78 H 3.94	$C_{20}H_{12}O_7$ C 65.61 H 3.47 C 65.94 H 3.32
FAB-MS ***m/z*** **(%)**	366 [M$^+$] (100)	364 [M$^+$] (100%)
UV (ethanol) λ_{max} **(ε) [nm]**	226 (68110), 299 (8770), 313 (6600), 328 (5460)	226 (72500), 298 (8220), 313 (5800), 327 (4620)
IR (KBr)	3640 (cw), 3460 (OH), 3400 (OH), 3050 (CH), 3020 (CH), 2920 (CH), 1695 (C=O), 1680 (C=O), 1640/1610/1585/ 1415/1380 (C=C naphthalene), 1275 (aryl-O)	3485 (OH), 3060 (CH), 3020 (CH), 2950 (CH), 1690 (C=O), 1640/1610/1585/ 1415/1380 (C=C naphthalene), 1275 (aryl-O)

Table 1: Physico-chemical properties of 1 and 2

	Chemical shifts δ [ppm] of 1				Chemical shifts δ [ppm] of 2		
Pos.	^{1}H NMR	*J* [Hz]	^{13}C NMR	^{1}*J* [Hz]	^{1}H NMR	*J* [Hz]	^{13}C NMR
1	-	-	188.7	-	-	-	190.0
2	5.84 (dd)	10.5; 1.0	125.3	166.8	6.65 (d)*	10.5	135.6
3	6.72 (dd)	10.5, 4.9	144.7	164.8	6.61 (d)*	10.5	136.4
4	4.72 (d)	4.9; 1.0	60.5	165.0	-	-	187.3
5	-	-	95.0	-	-	-	94.5
6	3.38 (d)	4.2	52.7*	181.5	3.55 (d)	4.1	55.2
7	3.43 (dd)	4.2; 3.0	55.2*	184.0	3.52 (dd)	4.1; 3.0	53.2
8	5.02 (d)	3.0	59.7	140.0	5.17 (dd)	7.1; 3.0	59.4
9	-	-	70.6	-	-	-	65.4
10	-	-	62.2	-	-	-	63.5
1'	-	-	145.4	-	-	-	144.9
2'	6.98 (d)	8.0	120.6	164.0	7.60 (d)	8.0	120.9
3'	7.47 (dd)	8.0	127.7	161.8	7.49 (dd)	8.0	127.8
4'	7.59 (d)	8.0	108.6	163.0	6.69 (d)	8.0	108.9
5'	7.60 (d)	8.0	109.0	163.0	7.14 (d)	8.0	109.2
6'	7.51 (dd)	8.0	127.8	161.8	7.53 (dd)	8.0	127.9
7'	7.06 (d)	8.0	120.7	164.0	7.62 (d)	8.0	121.0
8'	-	-	145.1	-	-	-	145.2
9'	-	-	111.5	-	-	-	111.5
10'	-	-	133.7	-	-	-	133.8
4-OH	5.94 (br.)	-	-	-	-	-	-
8-OH	5.61 (br.)	-	-	-	3.12 (d)	7.1	-

Table 2: NMR-Data of **1** and **2** in DMSO-d_6 (* = interchangeable)

Comparison of the NMR data of the compounds **1** and **2** suggested that these structures differ only in their oxidation levels. ^{1}H and ^{13}C NMR experiments validated that in **2** the 4-OH group was replaced by a carbonyl function. The ^{13}C NMR of **2** only showed one carbon, bearing a secondary alcohol (C-8) and two carbonyls (C-1, -4) at 189.9 and 187.2 ppm. According to the ^{1}H NMR [6.65 (d, 2-H), 6.61 (d, 3-H)], the oxidation product contained an endione fragment.

Despite all efforts, the stereochemistry of the epoxides and the hydroxyl groups could not be defined by spectroscopic methods. Therefore we proceeded to solve this problem by performing a single crystal structure analysis of **2** [3]. Compound **2** gave suitable crystals, which allowed to establish the configuration as shown in figure 2 with the following stereochemistry: C-8/H-8α, C-7/H-7α, C-6/H-6α.

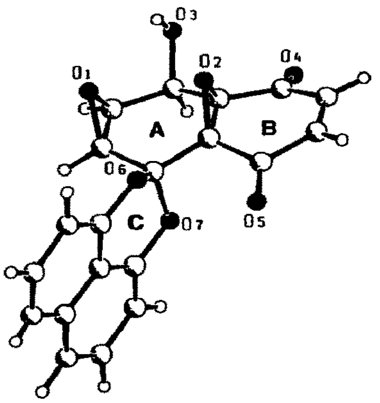


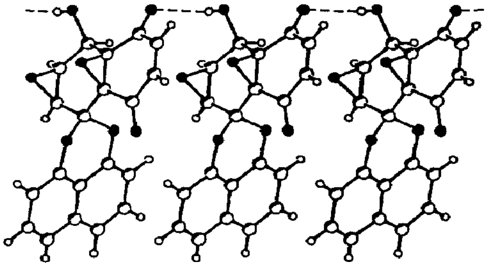
Figure 2: Schakal plot of **2**; the spatial representation with random absolute configuration is shown

O1···O2: 2.79 Å; C6O1C7: 61.6(4)°; C9O2C10: 62.5(3)°; C2-C3: 1.324(9); C9-C10: 1.500(7); C6-C7: 1.462(8); O1-C6: 1.414(7); O1-C7: 1.443(7); O2-C9: 1.456(7); O2-C10: 1.436(6)

Both the 4-OH group and the epoxides assume the *syn* configuration. The two six-membered rings A and B adopted a boat conformation; the ring junction between them was *cis*. Ring C had a envelope conformation. The molecules in the crystal were linked by intermolecular hydrogen bonds between O3-H and O4. Some van der Waals contacts were also perceived. The geometrical details of the H-bonds such as distances and angles are given in table 3.

H-bond	distances [Å]	H-bond	angles [°]
O3-H	0.60	O3-H···O4	173
H···O4	2.33	H···O4-C1	125
O3···O4	2.933		

Table 3: Intra and intermolecular hydrogen bonds



The X-ray analysis provided proof of the structure of **2**, which was suggested by several NMR experiments before. It simultaneously established the β-configuration for both the epoxides and the 8-hydroxyl group. The distance of 2.79 Å between the epoxides is noteworthy, since this amount is slightly larger than the sum of the van der Waals radii (2.60 Å). An assignment of the absolute configuration was not possible without a heavy atom. Unfortunately, the stereochemistry of 4-OH in **1** remains undefined. The solution of this problem will be reported later. Similar compounds were recently isolated from fermentation broths [5,6].

References and Notes

1) We thank Prof. Rampelli at the University of Viterbo (Italy) for providing us with cultures of this strain.
2) We are indebted to Prof. Emil Müller (CH-8702 Zollikon, Switzerland) for the taxonomic assignment.
3) F. Petersen, T. Moerker, F. Vanzanella, H. H. Peter, manuscript in preparation.

   Crystal structure analysis of **2**:
4) Crystallographic details: $C_{20}H_{12}O_7$, orthorhombic, space group $P2_12_12_1$, a = 5.498 (1) Å, b = 11.932 (1) Å, c = 24.131 (1) Å, Z = 4. A NONIUS CAD4 automatic diffractometer was used for data collection with CuKα radiation and graphite monochromator. The intensities of 1935 independent reflections with $\theta < 28°$ were measured, of which 1784 were classified as observed with $I > 2\sigma(I)$.

   The structure was solved by direct methods. All hydrogen atoms were found from a difference Fourier map. The structure was refined by fullmatrix least squares calculations with anisotropic (isotropic for hydrogen atoms) thermal parameters to a final R value of 0.064. Final fractional coordinates (bond length and angles) were deposited as sublementary material.

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6) G. Schlingmann, R. R. West, L. Milne, C. J. Pearce, G. T. Carter, *Tetrahedron Lett.* **1993**, *34*, 7225-7228.

(*Received in Germany* 17 *November* 1993; *accepted* 13 *December* 1993)