X-Ray Structure Analysis and Spectroscopic Data of the Antibiotic 8-(Dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone from the Fungus *Mollisia* sp.*

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Dedicated to Professor E. Hecker on the occasion of his 60th birthday

Naphthoquinone, X-Ray Structure Analysis, Mass Spectra, ^{13}C, ^{1}H NMR

Mass spectra, X-ray data and high resolution ^{13}C and ^{1}H NMR spectra of 8-(dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone are reported. The antibiotic active compound was isolated from the fungus *Mollisia* sp.

Introduction

During investigation of the mycoflora of dead branches of deciduous trees a dematiaceous hyphomycete was isolated by Butin and Kowalski [1] which shows strong antibiotic activity against the ascomycetes *Pezicula cinnamomea* (DC) *Sacc., Amphiporthe leiphaemia* (Fr. Butin), *Diaporthe carpini* (Fr.) Fckl. and against the basidiomycete *Heterobasidion annosum* (Fr.) Bref. (Kowalski, unpublished data).

Especially in dual cultures with *H. annosum* yellow crystals are produced. By detailed analytical investigations this compound could be identified as Mollisin which first was isolated by Gremmen [2] from *Mollisia* (Fr.) Karst. and characterized [3] as 8-(dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone, $C_{14}H_{10}Cl_2O_4$ (Fig. 1). In the course of our structural characterization of this compound a series of physicochemical data have been determined. The still undescribed X-ray structure analysis, the mass spectra and the high resolution ^{13}C and ^{1}H NMR data will be presented in this paper.

Experimental

The fungus *Mollisia* sp. is present in West Germany and the southern part of Poland during all seasons at small twigs and branches of many tree species. Stock cultures are maintained at Krakow (No.: HMIPC 15188, 15189, 15190).

On malt agar (20 g/l malt extract, 15 g/l Agar, Sigma, München, FRG) the growth rate is low. The fungus starts to form the yellow crystals only 10–14 days after inoculation around the colony (about 12 mm in diameter) in clusters of variable shape. The crystal formation occurs in monocultures, but is greatly enhanced in dual cultures, especially in combination with *Heterobasidion annosum*. Isolation was performed by extraction of the corresponding agar areas with $CHCl_3$ and successively by preparative thin layer chromatography on Kieselgel 60 (Merck, Darmstadt, FRG).

After recrystallization in ethanol the yellow compound showed decomposition at 196 °C.

UV spectra were recorded with a Perkin-Elmer λ 5 UV/VIS spectrophotometer. Mass spectra were recorded with a Varian/Finnigan CH7A + datasystem SS200 MS. ^{13}C and ^{1}H spectra were measured with a Bruker NMR spectrometer AM 500.

X-ray crystal structure analysis was performed with an Enraf-Nonius CAD4 Diffractometer. A crystal with dimensions $0.01 \times 0.05 \times 0.30$ mm, gained by recrystallization from $CHCl_3$ was used. With Mo-K_α

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* Further details of the investigations on crystal structures may be received at: "Fachinformationszentrum Energie, Physik, Mathematik, GmbH, D-7514 Eggenstein-Leopoldshafen 2". The Registrations-No., CSD 51981, the name of the author, and the reference should be given.

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radiation (graphite monochromator) 807 unique observed reflections with $|F_o| > 3\sigma(|F_o|)$ were collected up to $2\theta = 47°$ in an $\omega/2\theta$ scan mode.

The structure was solved by direct methods (290 $|E| > 1.1$) and refined by least squares methods (full matrix) with anisotropic thermal parameters for the heteroatoms and methyl C-atoms only [4]. All hydrogen atoms could be localized from a difference Fourier map. The hydroxyl H was treated as a riding atom, other H-atoms were included in calculated positions (C–H = 1.00 Å); idealized methyl groups were refined as rigid groups. All isotropic temperature parameters of the hydrogens were kept fixed at the corresponding values of the attached atoms. Omitting 7 reflections suffering from secondary extinction and machine fault, respectively, resulted in a final R-value of 0.062 and $R_w = 0.052$, $w^{-1} = \sigma^2(|F_o|) + 10^{-4}|F_o|^2$.

The lists of structure factors, positional and temperature parameters of all atoms, bond distances, bond angles and torsion angles have been deposited at the Fachinformationszentrum Energie – Physik – Mathematik, D-7514 Eggenstein-Leopoldshafen. Copies may be obtained by giving the deposition number CSD–51981, the names of the authors and the citation of this article.

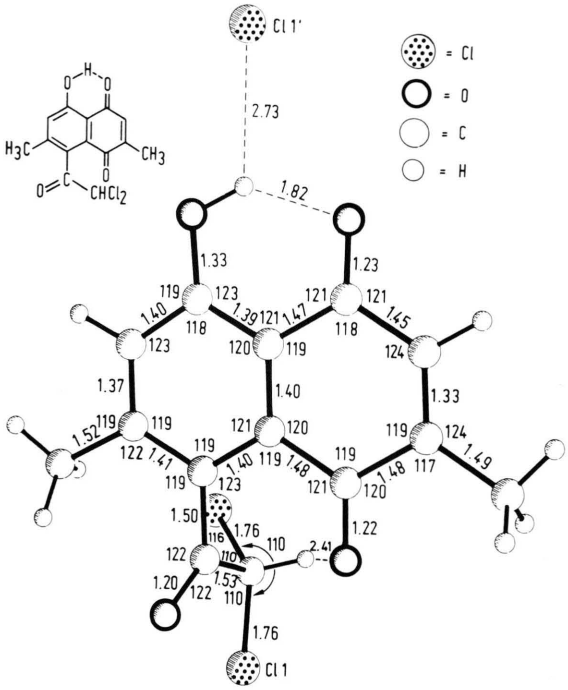

Fig. 1. Molecular structure of 8-(dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone in a perspectivity view. The distances between nonhydrogen atoms have standard deviations of 0.01 Å. The angles are given without the last significant digit (σ: 0.5–0.9°).

Results and Discussion

X-ray structure analysis

8-(Dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone crystallizes in the monoclinic space group $P2_1/c$; a = 5.061 (4), b = 15.797 (10), c = 16.570 (8) Å, β = 89.83 (5)°, V = 1324.7 Å^3, D_x = 1.570 Mg/m^3, Z = 4. Fig. 1 shows the molecular structure including bond distances and angles defined by nonhydrogen atoms. The naphthalene skeleton including the directly bonded atoms is planar in a first approximation. Due to sterical effects some small deviations from a strictly planar arrangement occur, which become obvious by differences of 7° as a maximum from the ideal values for the torsion angles (0° or 180° respectively). The OH-group forms a bifurcated hydrogen bridge: a relatively strong intramolecular one with the carbonyl group in *peri*-position and a weaker intermolecular bridge with one of the chlorine atoms of the dichloroacetyl group of a neighbouring molecule in the crystal structure. There also is a weak intramolecular H···O interaction between the H of the $CHCl_2$ group and a C=O group of the quinone.

The bond distances within the carbon skeleton of the 1,4-naphthoquinone system are quite normal. The benzene ring shows bond distances between 1.37 and 1.40 Å, in accordance with the intermediate bond orders for the aromatic ring system. The 1,4-*para*-quinoide ring system reveals three types of C-C-distances in accordance with the canonical formula: four bonds near single bond distances (1.45–1.48 Å), one near a double bond distance (1.33 Å) and that C-C-bond in common with the benzene ring (1.40 Å), whose distance is of intermediate bond order.

The *mass spectroscopic* data showing the fragmentation pattern of the described naphthoquinone derivative are listed in Table I.

Table I. Mass spectrum (70 eV); *m/z* 229 ≡ 100% rel. intensity.

	Fragments (Relative intensities)
314 (0.2)	173 (2.8)
312 (0.6)	145 (4.0)
277 (0.6)	128 (2.1)
231 (2.3)	115 (5.1)
230 (14.9)	105 (2.3)
229 (100)	91 (2.8)
214 (2.3)	77 (3.2)
201 (3.8)	51 (3.9)

The *UV spectrum* corresponds to that qualitatively described by van der Kerk and Overeem [5]: λmax [ε] in ethanol: 207 [22900]; 254 [16000]; (270) [10900]; 413 [2700].

Concerning *NMR data,* a rough proton resonance spectrum already has been measured [6]. In Table II data from highly resolved ^{1}H and ^{13}C NMR spectra are listed and compared to calculated shift values for control.

All described spectroscopic data and also the ^{13}C data in comparison to the calculated values fit best to the described structure. They should be interesting for documentation purposes.

With view to biological aspects the antibiotic activity (Kowalski, unpublished) of the described compound produced from *Mollisia* sp. is interesting. After inoculation the fungus may prevent trees from superinfections for instance by the highly pathogenic fungus *Heterobasidion annosum* one of the most destructive basidiomycete pathogens in the coniferous forests of the world. Up to now no evidence for a pathogenicity of *Mollisia* sp. by itself has been observed.

Table II. Data from ^{1}H and ^{13}C NMR spectra measured in CDCl$_3$ at 25°, TMS ≡ 0 ppm, ^{13}C data calculations were done according to [7].

	ppm (intensity)	Spin state	Coupling constant [Hz]
^{1}H: 3-H	6.84 (1)	q.	J = 1.5
6-H	7.19 (1)	q.	J = 0.4
(2)-CH	2.17 (3)	d.	J = 1.8
(7)-CH$_3$	2.44 (3)	d.	J = 0.4
OH	12.08 (1)	s.	
−CHCl$_2$	6.32 (1)	s.	

	found	calc.		found	calc.
^{13}C: 1-C	189.3 s	187.0	7-C	146.3 s	145.4
2-C	148.7 s	145.9	8-C	130.1 s	126.6
3-C	136.1 d	133.3	8′-C	130.3 s	135.5
4-C	186.0 s	187.0	−C=0	192.0 s	192.0
4′-C	113.0 s	116.2	CHCl$_2$	70.9 d	70.2
5-C	162.2 s	161.6	(7)-CH$_3$	20.5 q	22.3
6-C	126.0 d	120.7	(2)-CH$_3$	16.3 q	15.8

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