LACHNUMON AND LACHNUMOL A, NEW METABOLITES WITH NEMATICIDAL AND ANTIMICROBIAL ACTIVITIES FROM THE ASCOMYCETE *Lachnum papyraceum* (KARST.) KARST.

II. STRUCTURAL ELUCIDATION

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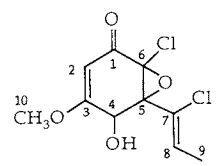
The structures of two new biologically active chlorinated metabolites isolated from submerged cultures of the ascomycete *Lachnum papyraceum* have been elucidated by NMR and mass spectroscopy. The compounds, lachnumon (**1**) and lachnumol A (**2**), which structurally are related with mycorrhizin A that also is produced by the fungus, contain an unusual chlorinated epoxide group.

In a screening of fungal extracts for metabolites with nematicidal activity, extracts of submerged cultures of the wood-inhabiting ascomycete *Lachnum papyraceum* showed high activity against *Caenorhabditis elegans*[1]. Five active metabolites were isolated[1], and these have been characterised essentially by spectroscopic methods. Three of the compounds have previously been reported in the literature, mycorrhizin A (**3**) and chloromycorrhizin A (**4**), which were isolated from a fungal source[2], and dechloromycorrhizin A (**5**), which is a synthetic product obtained during a total synthesis of mycorrhizin A[3], while lachnumon (**1**) and lachnumol A (**2**) are new natural products. From the MS data (see Table 1), both from HR measurements and the isotopic patterns observed, it is evident that both contain two chlorine each. The MS data, and also the UV and IR data, suggest that the difference between the two compounds may be that a carbonyl function in lachnumon (**1**) has been reduced to an alcohol in lachnumol A (**2**). This could be confirmed by reducing lachnumon (**1**) with sodium borohydride in ethanol. Although several products were formed, lachnumol A (**2**) could be isolated as a main product.



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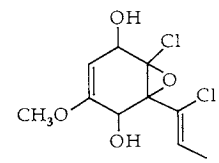

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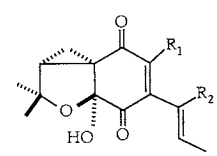
Lachnumol A (**2**) contains 2 double bonds according to the NMR data, while lachnumon (**1**) has an additional carbonyl function, which means that the compounds contain 2 rings. Three of the four oxygens could be accounted for by one methoxy and two hydroxy groups in lachnumol A (**2**) (one of the latter exists as a carbonyl oxygen in lachnumon



3 $R_1=H$, $R_2=Cl$
4 $R_1=Cl$, $R_2=Cl$
5 $R_1=H$, $R_2=H$

Table 1. Physico-chemical properties of lachnumon (**1**) and lachnumol A (**2**).

	Lachnumon (**1**)	Lachnumol A (**2**)
Appearance	Colorless crystals	Colorless oil
MP (°C)	70 (decomp.)	
$[\alpha]_D^{22}$	+75° (*c* 5 in $CHCl_3$)	−6.5° (*c* 2 in $CHCl_3$)
Molecular formula	$C_{10}H_{10}O_4Cl_2$	$C_{10}H_{12}O_4Cl_2$
HREI-MS (*m/z*)		
Observed	263.9946 (M^+)	266.0133 (M^+)
Calculated	263.9956 for $C_{10}H_{10}O_4{}^{35}Cl_2$	266.0112 for $C_{10}H_{12}O_4{}^{35}Cl_2$
EI-MS	268 (10% of 264), 266 (70% of 264), 264 (1%), 237 (65% of 235), 235 (3%), 231 (35% of 229), 229 (5%), 221 (70% of 219), 219 (4%), 203 (35% of 201), 201 (100%)	270 (10% of 266), 268 (65% of 266), 266 (13%), 253 (15% of 249), 251 (65% of 249), 249 (16%), 223 (15% of 219), 221 (65% of 219), 219 (38%), 217 (2% of 213), 215 (35% of 213), 213 (100%)
UV (MeOH)		
λ_{max} nm (ε)	260 (4,500)	209 (4,800)
IR (KBr) cm^{-1}	3420, 1655, 1600, 1240	3400, 1670, 1225, 915, 740
TLC (Rf)	0.54[a], 0.50[b]	0.41[a], 0.47[b]

[a] Merck, Kieselgel 60 F_{254}: Toluene - aceton - AcOH (70 : 30 : 1).
[b] Merck, Kieselgel 60 F_{254}: Toluene - ethyl formate - formic acid (10 : 5 : 3).

Table 2. ^1H and ^{13}C NMR data of lachnumon (**1**) and lachnumol A (**2**) in $CDCl_3$ (300 MHz and 75 MHz, respectively).

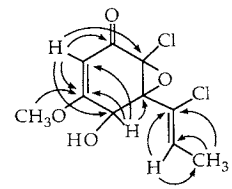
Proton No.	**1**	**2**	Carbon No.	**1**	**2**
1-H	—	4.72 (dd, 2.9, 5.9)	C-1	183.4 (s)	71.5 (d)
2-H	5.42 (s)	4.64 (d, 2.9)	C-2	97.5 (d)	95.1 (d)
4-H	5.11 (d, 4.9)	4.84 (d, 2.6)	C-3	171.3 (s)	153.0 (s)
8-H	6.20 (q, 6.8)	6.13 (q, 6.8)	C-4	65.0 (d)	64.2 (d)
9-H_3	1.89 (d, 6.8)	1.86 (d, 6.8)	C-5	68.2 (s)	69.5 (s)
10-H_3	3.80 (s)	3.60 (s)	C-6	80.5 (s)	84.0 (s)
1-OH	—	2.57 (m)	C-7	124.3 (s)	125.2 (s)
4-OH	2.78 (d, 4.9)	2.65 (m)	C-8	128.1 (d)	127.3 (d)
			C-9	13.7 (q)	13.6 (q)
			C-10	56.9 (q)	55.2 (q)

The chemical shifts are given in ppm relative TMS, and the multiplicity and the coupling constant(s) (in Hz) are given in parenthesis.

(**1**)). The NMR analyses were complicated by the decomposition of both compounds in common NMR solvents during prolonged experiments at room temperature. However, by using the quality of $CDCl_3$ indicated in the experimental section and filtering it through a plug of Na_2CO_3 immediately before use, the problem could be handled. The NMR data of compounds **1** and **2** are given in Table 2, and it is obvious that limited structural information is obtained from interproton couplings. An additional coupling between 2-H and 4-H in both compounds could be revealed by COSY experiments. Instead, long range heteronuclear couplings observed both by 2D techniques and by the recording of coupled ^{13}C NMR spectra with selective irradiation of pertinent proton frequencies were essential for the determination of the structures. Especially important was the observation that irradiation of 2-H affect the fine splitting of C-6 (in lachnumon (**1**) a 7 Hz splitting collapse into a singlet), as this establishes a link between C-1 and C-6 that otherwise was difficult to obtain. Irradiation of 2-H in lachnumon (**1**) also affected the signals

for C-1, C-3, and C-4, while irradiation of 4-H affected the signals for C-2, C-3, and C-5. All observed long range heteronuclear couplings for lachnumon (**1**) are summarised in Fig. 1.

Fig. 1. Long range heteronuclear couplings observed with lachnumon (**1**).



NOE experiments with lachnumol A (**2**) revealed the C-7/C-8 double bond to be the *Z* isomer, as irradiation of 8-H resulted in a small NOE on 4-H (1.5% enhancement), while irradiation of 9-H_3 gave no effect. However, this and other NOE's are not sufficient to determine the relative configuration of, for instance, C-4 and C-5. Dreiding models of any of the possible isomers of the two compounds indicate that they may exist in several conformations, and in the absence of reliable methods to calculate their relative steric energy (reliable parameters for some of the unusual combinations of atoms are missing), NOE data can not be used to discriminate between the possible alternatives. Attempts to prepare derivatives that could be useful in this respect failed, largely because of the chemical instability of the compounds and the limited amounts available. They are especially sensitive to traces of acid, and the number of compounds formed in for example normal grade $CDCl_3$ suggest that the epoxide ring readily opens to a cation that reacts further. In addition to lachnumon (**1**) and lachnumol A (**2**), an unseparatable mixture of two compounds believed to be diastereomers of lachnumol A (**2**) was also obtained from the extracts. As different fermentations yielded slightly different ratios of the products, it was possible to determine which signals in spectra of the mixture belongs to which of the two components, and their NMR data are very similar to those of lachnumol A (**2**).

Lachnumon (**1**) and lachnumol A (**2**) are obviously related to mycorrhizin A (**3**) which in turn belongs to a group of antifungal metabolites with a pentaketide origin[4], although it is unlikely that lachnumon (**1**) or lachnumol A (**2**) are precursors of mycorrhizin A (**3**) in *Lachnum papyraceum*. Epoxides in which one of the epoxide carbons is chlorinated are scarce as natural products (none is listed in ref 5), but have previously been isolated as cyclic nucleotide phosphodiesterase inhibitors from the fungus *Mollisia ventosa*[6].

Experimental

General

The compounds were isolated from the organic extract of a culture filtrate of the fungus *Lachnum papyraceum*[1]. UV spectra were obtained with a Perkin-Elmer λ16, and IR spectra with a Bruker IFS 48. The optical rotation was measured with a Perkin-Elmer 1541 polarimeter with a cell path of 10 cm. EI-MS and HREI-MS spectra (direct inlet, EI at 70 eV) were recorded with a JEOL JMS-SX102 spectrometer, and NMR spectra ($CDCl_3$), were obtained with a Varian XL-300 spectrometer. The $CDCl_3$ (99.95% isotopic purity and stabilized with silver) used in the NMR experiments was obtained from Glaser AG (Switzerland) in 25 ml bottles. TLC experiments were performed on Merck Kieselgel 60 F_{254} precoated plates, and LC separations were performed on a Merck Lobar prepacked silica gel column.

Reduction of Lachnumon (**1**) to Lachnumol A (**2**)

To a solution of 5 mg of **1** in 1 ml of ethanol, 3 mg of finely divided sodium borohydride was added. The suspension was stirred for 5 minutes whereafter it was filtrated and the solvent was evaporated. Approximately 1 mg of lachnumol (**2**) was obtained after chromatography on silica gel.

Acknowledgment

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References

1) STADLER, M.; H. ANKE, W.-R. ARENDHOLZ, F. HANSSKE, U. ANDERS, O. STERNER & K.-E. BERGQUIST: Lachnumon and lachnumol A, new metabolites with nematicidal and antimicrobial activities from the ascomycete *Lachnum papyraceum* (Karst.) Karst. I. Producing organism, fermentation, isolation and biological activities. J. Antibiotics 46: 961~967, 1993
2) TROFAST, J. & B. WICKBERG: Mycorrhizin A and dechloromycorrhizin A, two antibiotics from a mycorrhizal fungus of *Monotropa hypopitys* L. Tetrahedron 38: 875~879, 1976
3) KOFT, E. B. & A. B. SMITH: Total synthesis of (±)-mycorrhizin A and (±)-dechlromycorrhizin A. J. Am. Chem. Soc. 104: 2659~2621, 1982
4) TURNER, W. B. & D. C. ALDRIDGE: Fungal metabolites II. pp. 82~93. Academic Press, London, 1983
5) Dictionary of Natural Products. Chapman and Hall Chemical Database, version 1:1 July 1992. Chapman and Hall, London, 1992
6) NAKANISHI, S.; K. ANDO, I. KAWAMOTO, T. YASUZAWA, H. SANO & H. KASE: KS-540 compounds, novel inhibitors of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase from *Mollisia ventosa*. J. Antibiotics 42: 1775~1783, 1989