New Metabolites with Nematicidal and Antimicrobial Activities from the Ascomycete *Lachnum papyraceum* (Karst.) Karst

VII. Structure Determination of Brominated Lachnumon and Mycorrhizin A Derivatives

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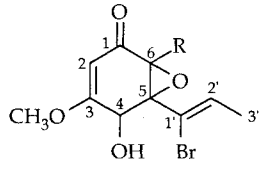
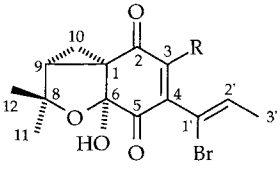
The structure determination of lachnumon B1 (**16**) and lachnumon B2 (**17**), brominated derivatives of lachnumon (**1**), as well as mycorrhizin B1 (**18**) and mycorrhizin B2 (**19**), brominated derivatives of mycorrhizin A (**3**), is described. The compounds, which exhibit similar antimicrobial and nematicidal activity as their chlorinated analogues, were isolated from extracts of cultures of the ascomycete *Lachnum papyraceum* to which $CaBr_2$ had been added. The structures were elucidated by spectroscopic methods.

Investigations of the production of halogenated metabolites by the ascomycete *Lachnum papyraceum* have shown that bromine can be incorporated by adding $CaBr_2$ to the culture medium, but the time of the $CaBr_2$ addition is important. If it is added at the beginning of the fermentation, only small amounts the normal metabolites **1**~**5** and **12**[1,2] and their brominated analogues are formed, but instead the chlorinated and brominated isocoumarins **6**~**11** are obtained as the major products[3,4] (the structures of all compounds **1**~**19** are given in the preceding paper). If $CaBr_2$ is added at a later stage, when the production of secondary metabolites has started, enhanced amounts of the papyracons **13**~**15** are formed together with the four new brominated lachnumon and mycorrhizin A derivatives **16**~**19**. The preceding two papers describe the isolation of the 8 new bioactive metabolites **13**~**19** from submerged cultures of the fungus to which $CaBr_2$ was added at the onset of the secondary metabolism[5], and the structure determination of four non-halogenated metabolites structurally related to mycorrhizin A (**3**)[6]. In this paper we describe the determination of the structures of lachnumon B1 (**16**), lachnumon B2 (**17**), mycorrhizin B1 (**18**) and mycorrhizin B2 (**19**).



16: R=H
17: R=Cl



18: R=H
19: R=Cl

Structure Determination of Lachnumon B1 (**16**)

In the EI-MS spectrum of lachnumon B1 (**16**) both M^+ and the base peak are doubled, suggesting the presence of bromine, and confirmed by high resolution measurements. The suggested molecular composition is $C_{10}H_{11}O_4Br$ (see Table 1), corresponding to a compound with 5 rings and/or unsaturations, and a peak for M−Br (*m/z* 195) is also observed. The fact that the compound contains 11 hydrogens is supported by the ^1H NMR spectrum (see Table 2), and 10 signals are present in the ^{13}C NMR spectrum (see Table 3). The structure was essentially determined by the long-range ^1H-^{13}C heteronuclear correlations (shown in Fig. 1) observed in a HMBC experiment. Compared to lachnumon (**1**), the structure determination is facilitated by the presence of 6-H which correlates to C-1 and C-5 in the HMBC spectrum. Together with the observed correlations for 2-H and 4-H, the 4-hydroxy-3-methoxy-cyclohex-2-enone system can be determined, and the attachment of the 1-propenyl group to C-5 is established by the HMBC-correlation between 1'-H and C-5. One oxygen, which must be part of an oxirane or oxetane

Table 1. Physico-chemical properties of compounds **16**, **17**, **18** and **19**.

	16	**17**	**18**	**19**
Appearance	Yellowish oil	Colourless crystals	Yellowish oil	Yellowish oil
MP (°C)	—	125 (decomp.)	—	—
$[\alpha]_D^{22}$	+214° (*c* 0.3 in $CHCl_3$)	+85° (*c* 0.3 in $CHCl_3$)	+29° (*c* 0.5 in $CHCl_3$)	+37° (*c* 0.1 in $CHCl_3$)
Molecular formula	$C_{10}H_{11}O_4Br$	$C_{10}H_{10}O_4ClBr$	$C_{14}H_{15}O_4Br$	$C_{14}H_{14}O_4ClBr$
HREI-MS (*m/z*)				
Observed	273.9811 (M^+)	307.9473 (M^+)	326.0178 (M^+)	341.9642 ($M^+ - H_2O$)
Calculated	273.9841 for $C_{10}H_{11}O_4{}^{79}Br$	307.9451 for $C_{10}H_{10}O_4{}^{35}Cl^{79}Br$	326.0154 for $C_{14}H_{15}O_4{}^{79}Br$	341.9659 for $C_{14}H_{12}O_3{}^{35}Cl^{79}Br$
EI-MS	276 (95% of 274), 274 (18%), 247 (96%), 245(100%), 231 (52%), 229 (53%), 195 (72%), 133 (74%), 69 (86%)	312 (30% of 308), 310 (130% of 308), 308 (2%), 283 (30% of 279), 281 (130% of 279), 279 (4%), 275, 273 (5%), 267 (30% of 263), 265 (130% of 263), 263 (2%), 247 (97%), 245 (100%), 201 (25%)	328 (130% of 326), 326 (3%), 310, 308 (30%), 295, 293 (5%), 267, 265 (6%), 247 (7%), 229 (52%), 201 (66%), 93 (78%), 65 (74%), 43 (100%)	364 (1%), 362 (2%), 360 (M^+, 1%), 346 (30% of 342), 344 (130% of 342), 342 (16%), 325, 327 (4%), 318 (40% of 314), 316 (135% of 314), 314 (21%), 299 (8%), 281 (16%), 263 (35%), 235 (65%), 207 (65%), 202 (84%), 173 (100%)
UV (MeOH)				
λ_{max} nm (ε)	259 (7,670)	261 (14,600)	233 (6,470), 303 (4,370)	252 (8,280), 308 (1,300)
IR (KBr) cm^{-1}	3440, 2980, 1665, 1615, 1385, 1220, 1185, 1050, 1020	3450, 2990, 1665, 1600, 1390, 1225, 840, 795	3450, 2980, 1715, 1665, 1570, 1395, 1320, 1285, 1175	3410, 2980, 1695, 1570, 1375, 1260, 1180, 1105, 1020
TLC (Rf)	0.42[a], 0.47[b]	0.49[a], 0.54[b]	0.56[a], 0.65[b]	0.57[a], 0.66[b]

[a] Merck, Kieselgel 60 F254: Toluene - aceton - AcOH (70 : 30 : 1).
[b] Merck, Kieselgel 60 F254: Toluene - ethyl formiat - formic acid (10 : 5 : 3).

Table 2. ^1H NMR data (500 MHz) of compounds **16**, **17**, **18** and **19**. The spectra were recorded in $CDCl_3$, the solvent signal (7.26 ppm) was used as reference, and the coupling constants are given in Hz.

Compound proton	**16**	**17**	**18**	**19**
2-H	5.26 (d; 1.5)	5.42 (s)	—	—
3-H	—	—	7.11 (s)	—
4-H	4.90 (d; 4)	5.18 (d; 5.7)	—	—
6-H	3.76 (d; 1.5)	—	—	—
9-H		—	2.23 (dd; 5.9, 8.3)	2.29 (dd; 6.0, 8.3)
10-Hα	—	—	1.58 (dd; 4.8, 5.9)	1.74 (dd; 5.0, 6.0)
10-Hβ	—	—	1.92 (dd; 4.8, 8.3)	2.06 (dd; 5.0, 8.3)
11-H_3	—	—	1.34 (s)	1.34 (s)
12-H_3	—	—	1.25 (s)	1.25 (s)
2′-H	6.34 (q; 6.6)	6.43 (q; 6.7)	7.03 (q; 6.7)	6.10 (q; 6.6)
3′-H_3	1.95 (dd; 1.7, 6.8)	1.89 (d; 6.7)	2.02 (d; 6.7)	1.97 (d; 6.6)
3-OCH_3	3.75 (s)	3.80 (s)	—	—
4-OH	2.62 (d; 4)	2.62 (d; 5.7)	—	—
6-OH	—	—	3.22 (br s)	3.25 (br s)

ring in order to account for the missing unsaturation, and one bromine now remains, and the chemical shifts for C-1′ and C-2′ indicate that no oxygen is bound to C-1′ which leaves structure **16** as the only alternative. Besides a strong NOESY correlation between 6-H and 2′-H, which shows that the 1′/2′ double bond is *Z* as in lachnumon (**1**), no conclusive evidence for the relative stereochemistry of lachnumon B1 (**16**) could be obtained.

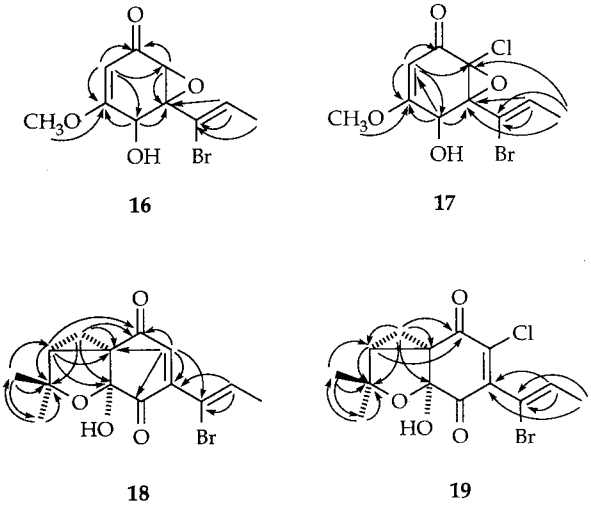
Structure Determination of Lachnumon B2 (**17**)

The EI-MS of lachnumon B2 (**17**) shows a different isotope pattern compared to lachnumon B1 (**16**), typical for the presence of one chlorine and one bromine[7]. The fragmentation is very similar to that of lachnumon (**1**)[2], with small peaks for M−29, M−35 and M−45, and M−63 as the base peak. The molecular composition, suggested by high resolution measurements, is $C_{10}H_{10}ClBr$,

Table 3. ^{13}C NMR data (125 MHz) of compounds **16**, **17**, **18** and **19**. The spectra were recorded in $CDCl_3$, and the solvent signal (77.0 ppm) was used as reference.

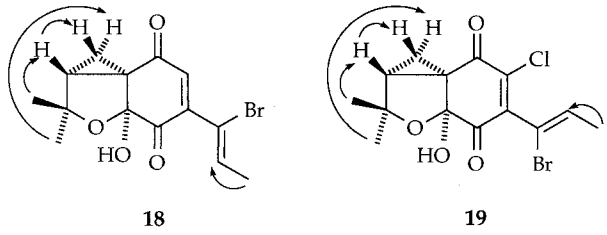
Carbon No.	16	17	18	19
C-1	192.4 (s)	183.3 (s)	43.1 (s)	42.3 (s)
C-2	98.2 (d)	97.5 (d)	192.3 (d)	185.1 (s)
C-3	172.2 (s)	171.1 (s)	137.9 (d)	145.0 (s)
C-4	66.5 (d)	65.4 (d)	146.2 (s)	146.4 (s)
C-5	65.8 (s)	68.7 (s)	191.7 (s)	189.7 (s)
C-6	60.6 (d)	80.8 (s)	101.0 (s)	99.6 (s)
C-8	—	—	82.8 (s)	82.6 (s)
C-9	—	—	44.8 (d)	46.2 (d)
C-10	—	—	14.8 (t)	15.4 (t)
C-11	—	—	25.0 (q)	24.7 (q)
C-12	—	—	29.1 (q)	29.3 (q)
C-1′	120.6 (s)	116.5 (s)	119.4 (s)	112.3 (s)
C-2′	132.3 (d)	131.4 (d)	139.9 (d)	133.6 (d)
C-3′	16.8 (q)	16.4 (q)	19.4 (q)	17.2 (q)
3-OCH_3	56.5 (q)	56.8 (q)		

Fig. 1. Significant ^1H-^{13}C long-range correlations observed for compounds **16**, **17**, **18** and **19**.



and this was confirmed by ^1H and ^{13}C NMR spectroscopy. The long-range ^1H-^{13}C correlations observed are summarized in Fig. 1, and after comparing the NMR data with those of lachnumon (**1**) it is obvious that the two compounds are very similar. The difference is that one of the chlorine atoms in lachnumon (**1**) has been replaced by a bromine in lachnumon (**17**), and the position of this bromine is deduced from the ^{13}C NMR data of the two compounds. Only the shift for C-1′ differ significantly between the two compounds, 124.3 ppm in **1** and 116.5 ppm in **17**, which is a typical shift change when changing from a chlorinated to a brominated sp^2 carbon[8]. The shift for the second halogenated carbon (C-6) is almost identical (80.5 ppm in **1** and 80.8 ppm in **17**) in the two compounds.

Fig. 2. Major NOESY correlations observed for compounds **18** and **19**.



Structure Determination of Mycorrhizin B1 (18)

The EI-MS spectrum of mycorrhizin B1 (**18**) shows twin peaks for M^+ and $M-H_2O$, typical for compounds containing one bromine. The fragment ions observed are obtained after the loss of H_2O, CH_3, CO and Br, and the molecular composition suggested by high resolution measurements is $C_{14}H_{15}O_4Br$. The spectral data for mycorrhizin B1 show large similarities with those of mycorrhizin A (**3**)[9], and NMR spectroscopy confirmed that the two compounds only differ in the halogen atom. The ^1H and ^{13}C NMR data are given in Tables 2 and 3, and significant ^1H-^{13}C long-range correlations are shown in Fig. 1. The relative stereochemistry of mycorrhizin B1 (**18**) was determined by NOE and NOESY experiments (the correlations observed are shown in Fig. 2), and the similarities of the NMR data with those of mycorrhizin A (**3**) (except for the ^{13}C NMR shifts for the brominated carbon and its neighbours). No NOE correlations to 3-H were observed, neither from 2′-H (the NOESY experiment was also recorded in C_6D_6 in which the signals for 3-H and 2′-H were separated by 0.33 ppm) nor from 3′-H_3. This suggests that the preferred conformation of mycorrhizin B1 (**18**) (and mycorrhizin A (**3**)) is as shown in Fig. 2, and is further discussed below.

Structure Determination of Mycorrhizin B2 (19)

A typical Cl_1Br_1 isotope pattern for mycorrhizin B2 (**19**) was observed in the EI-MS spectrum. The molecular ion was weak and not suitable for high resolution measurements, but the exact mass of $M-H_2O$ corresponds to the composition $C_{14}H_{12}O_3ClBr$. The fragments formed after the loss of H_2O, Cl, H_2O+CO, $H_2O+CO+CH_3$, Br, $Br+H_2O$, $Br+H_2O+CO$, and $Br+H_2O+CO+CO$ were observed. The corresponding fragmentation has been reported for chloromycorrhizin A (**4**)[9], and besides the ^{13}C NMR shifts of C-4, C-1′, C-2′ and C-3′ the NMR data of the two compounds are almost identical (*vide supra*). The ^1H-^{13}C long-range and

NOESY correlations are summarized in Figs. 1 and 2. Due to the small amounts of the compound available for spectroscopy, it was not possible to observe weak ^{1}H-^{13}C long-range correlations, and no correlations to C-3 and C-5 were observed. However, all spectral data, including UV and IR data, are in agreement with the structure **19**, and comparable with the data for chloromycorrhizin A (**4**) for which a crystallographic analysis has been undertaken[10]. It is interesting to note the difference in the ^{1}H NMR shift for 2'-H between mycorrhizin B1 (**18**) and mycorrhizin B2 (**19**), and between mycorrhizin A (**3**) and dechloromycorrhizin A (**4**). The change is approximately 1 ppm (upfield) when a chlorine atom is introduced at C-3, indicating that steric interactions prevent the conjugation of the C-1'~C-2' double bond with the 1,4-diketocyclohex-2-en system.

The general interest in halogenated natural products has increased rapidly in the last years, partly due to their biological activities but also because of their apparent ecological significance in natural chemical defense systems[11]. The brominated metabolites isolated from *Lachnum papyraceum* in this investigation are further examples of how bromine can be introduced into normally chlorinated fungal metabolites when bromide salts are added to the culture medium. Besides obtaining derivatives that are useful for QSAR studies and for assessing the importance of the halogen atom for the biological activity of the metabolites, the shifts in the secondary metabolism of the fungus induced by the addition of bromide to the culture medium may also be helpful during studies of the biosynthesis of the mycorrhizins.

Experimental

The compounds were isolated from the organic extract of a culture filtrate of the fungus *Lachnum papyraceum*[5]. UV spectra were obtained with a Perkin Elmer λ 16, 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 (in $CDCl_3$ and C_6D_6) were recorded with a Bruker ARX500 spectrometer. TLC experiments were performed on Merck Kieselgel 60 F_{254} precoated plates.

Acknowledgments

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