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

VII. Structure Determination of Brominated Lachnumon
and Mycorrhizin A Derivatives

Marc Stadler and Heidrun Anke
University of Kaiserslautern, Department of Biotechnology,
Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany

Olov Sterner*
University of Lund, Department of Organic Chemistry 2, Chemical Center,
P.O.B. 124, S-221 00 Lund, Sweden

(Received for publication August 16, 1994)

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 CaBr₂ 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
CaBr₂ to the culture medium, but the time of the CaBr₂
addition is important. If it is added at the beginning of
the fermentation, only small amounts the normal meta-
bolites 1~5 and 12~12) and their brominated analogues
are formed, but instead the chlorinated and brominated
isocoumarins 6~11 are obtained as the major prod-
ucts3,4) (the structures of all compounds 1~19 are given
in the preceding paper). If CaBr₂ 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 CaBr₂ was added at the onset of
the secondary metabolism5), and the structure deter-
mination of four non-halogenated metabolites structur-
ally 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).

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
C₁₀H₁₁O₄Br (see Table 1), corresponding to a com-
ponent 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 ¹³C NMR spectrum (see Table 3). 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 ¹³C NMR spectrum (see Table 3). The
structure was essentially determined by the long-range
¹H,¹³C 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

\[ \text{CH}_3O \]
\[ \text{OH} \quad \text{Br} \]
\[ 16: R=H \]
\[ 17: R=Cl \]

\[ \text{HO} \quad \text{O} \quad \text{Br} \]
\[ 18: R=H \]
\[ 19: R=Cl \]
Table 1. Physico-chemical properties of compounds 16, 17, 18 and 19.

<table>
<thead>
<tr>
<th>Appearance</th>
<th>MP (°C)</th>
<th>MS2</th>
<th>Molecular formula</th>
<th>HREI-MS (m/z)</th>
<th>Observed</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Yellowish oil</td>
<td>+214° (c 0.3 in CHCl3)</td>
<td>C10H11O4Br</td>
<td>273.9811 (M+)</td>
<td>273.9841</td>
<td>273.9811 (M+)</td>
</tr>
<tr>
<td>17</td>
<td>Colourless crystals</td>
<td>+85° (c 0.3 in CHCl3)</td>
<td>C10H11O4ClBr</td>
<td>276 (95%) of 274, 274</td>
<td>276 (95%) of 274, 274</td>
<td>276 (95%) of 274, 274</td>
</tr>
<tr>
<td>18</td>
<td>Yellowish oil</td>
<td>+29° (c 0.5 in CHCl3)</td>
<td>C14H15O4Br</td>
<td>297 (99%) of 297</td>
<td>297 (99%) of 297</td>
<td>297 (99%) of 297</td>
</tr>
<tr>
<td>19</td>
<td>Yellowish oil</td>
<td>+37° (c 0.1 in CHCl3)</td>
<td>C14H14O4ClBr</td>
<td>307 (99%) of 307</td>
<td>307 (99%) of 307</td>
<td>307 (99%) of 307</td>
</tr>
</tbody>
</table>

UV (MeOH)

<table>
<thead>
<tr>
<th>λmax nm (ε)</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowish oil</td>
<td>259 (7,670)</td>
<td>261 (14,600)</td>
<td>233 (6,470)</td>
<td>252 (8,280)</td>
</tr>
</tbody>
</table>

Table 2. 1H NMR data (500 MHz) of compounds 16, 17, 18 and 19. The spectra were recorded in CDCl3, the solvent signal (7.26 ppm) was used as reference, and the coupling constants are given in Hz.

<table>
<thead>
<tr>
<th>Compound proton</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-H</td>
<td>5.26 (d; 1.5)</td>
<td>5.42 (s)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3-H</td>
<td>—</td>
<td>—</td>
<td>7.11 (s)</td>
<td>—</td>
</tr>
<tr>
<td>4-H</td>
<td>4.90 (d; 4)</td>
<td>5.18 (d; 5.7)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6-H</td>
<td>3.76 (d; 1.5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9-H</td>
<td>—</td>
<td>—</td>
<td>2.23 (dd; 5.9, 8.3)</td>
<td>2.29 (dd; 6.0, 8.3)</td>
</tr>
<tr>
<td>10-Hx</td>
<td>—</td>
<td>—</td>
<td>1.58 (dd; 4.8, 5.9)</td>
<td>1.74 (dd; 5.0, 6.0)</td>
</tr>
<tr>
<td>10-Hβ</td>
<td>—</td>
<td>—</td>
<td>1.92 (dd; 4.8, 8.3)</td>
<td>2.06 (dd; 5.0, 8.3)</td>
</tr>
<tr>
<td>11-H3</td>
<td>—</td>
<td>—</td>
<td>1.34 (s)</td>
<td>1.34 (s)</td>
</tr>
<tr>
<td>12-H3</td>
<td>—</td>
<td>—</td>
<td>1.25 (s)</td>
<td>1.25 (s)</td>
</tr>
<tr>
<td>2'-H</td>
<td>6.34 (q; 6.6)</td>
<td>6.43 (q; 6.7)</td>
<td>7.03 (q; 6.7)</td>
<td>6.10 (q; 6.6)</td>
</tr>
<tr>
<td>3'-H3</td>
<td>1.95 (dd; 1.7, 6.8)</td>
<td>1.89 (d; 6.7)</td>
<td>2.02 (d; 6.7)</td>
<td>1.97 (d; 6.6)</td>
</tr>
<tr>
<td>3-OCH3</td>
<td>3.75 (s)</td>
<td>3.80 (s)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4-OH</td>
<td>2.62 (d; 4)</td>
<td>2.62 (d; 5.7)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6-OH</td>
<td>—</td>
<td>—</td>
<td>3.22 (brs)</td>
<td>3.25 (brs)</td>
</tr>
</tbody>
</table>

rung 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 bromine7. The fragmentation is very similar to that of lachnumon (1), 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 C16H10ClBr.
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.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>192.4 (s)</td>
<td>183.3 (s)</td>
<td>43.1 (s)</td>
<td>42.3 (s)</td>
</tr>
<tr>
<td>C-2</td>
<td>98.2 (d)</td>
<td>97.5 (d)</td>
<td>192.3 (d)</td>
<td>185.1 (s)</td>
</tr>
<tr>
<td>C-3</td>
<td>172.2 (s)</td>
<td>171.1 (s)</td>
<td>137.9 (d)</td>
<td>145.0 (s)</td>
</tr>
<tr>
<td>C-4</td>
<td>66.5 (d)</td>
<td>65.4 (d)</td>
<td>146.2 (s)</td>
<td>146.4 (s)</td>
</tr>
<tr>
<td>C-5</td>
<td>65.8 (s)</td>
<td>68.7 (s)</td>
<td>191.7 (s)</td>
<td>189.7 (s)</td>
</tr>
<tr>
<td>C-6</td>
<td>60.6 (d)</td>
<td>80.8 (s)</td>
<td>101.0 (s)</td>
<td>99.6 (s)</td>
</tr>
<tr>
<td>C-8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C-9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C-10</td>
<td>—</td>
<td>—</td>
<td>25.0 (q)</td>
<td>24.7 (q)</td>
</tr>
<tr>
<td>C-11</td>
<td>—</td>
<td>—</td>
<td>29.1 (q)</td>
<td>29.3 (q)</td>
</tr>
<tr>
<td>C-12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C-1'</td>
<td>120.6 (s)</td>
<td>116.5 (s)</td>
<td>119.4 (s)</td>
<td>112.3 (s)</td>
</tr>
<tr>
<td>C-2'</td>
<td>132.3 (d)</td>
<td>131.4 (d)</td>
<td>139.9 (d)</td>
<td>133.6 (d)</td>
</tr>
<tr>
<td>C-3'</td>
<td>16.8 (q)</td>
<td>16.4 (q)</td>
<td>19.4 (q)</td>
<td>17.2 (q)</td>
</tr>
<tr>
<td>3-OCH$_3$</td>
<td>56.5 (q)</td>
<td>56.8 (q)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

and this was confirmed by $^1$H and $^{13}$C NMR spectroscopy. The long-range $^1$H-$^{13}$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. 1. Significant $^1$H-$^{13}$C long-range correlations observed for compounds 16, 17, 18 and 19.

![Fig. 1](image)

Structure Determination of Mycorrhizin Bl (18)

The EI-MS spectrum of mycorrhizin Bl (18) shows twin peaks for M$^+$ and M – H$_2$O, typical for compounds containing one bromine. The fragment ions observed are obtained after the loss of H$_2$O, CH$_3$, CO and Br, and the molecular composition suggested by high resolution measurements is C$_{14}$H$_{15}$O$_4$Br. The spectral data for mycorrhizin Bl 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 $^1$H and $^{13}$C NMR data are given in Tables 2 and 3, and significant $^1$H-$^{13}$C long-range correlations are shown in Fig. 1. The relative stereochemistry of mycorrhizin Bl (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$_6$D$_6$ 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 Bl (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$_x$Br$_y$ 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$_2$O corresponds to the composition C$_{14}$H$_{12}$O$_3$ClBr. The fragments formed after the loss of H$_2$O, Cl, H$_2$O + CO, H$_2$O + CO + CH$_3$, Br, Br + H$_2$O, Br + H$_2$O + CO, and Br + H$_2$O + 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 $^1$H-$^{13}$C long-range and
NOEY 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 \textit{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 \textit{Lachnum papyraceum}\(^ 5\). UV spectra were obtained with a Perkin Elmer \(^{16}\) 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\(_6\)D\(_6\)) were recorded with a Bruker ARX500 spectrometer. TLC experiments were performed on Merck Kieselgel 60 F\(_{254}\) precoated plates.

**Acknowledgments**

Financial support from the Swedish Natural Science Research Council is gratefully acknowledged.

**References**


2) Stadler, M.; H. Anke, K. E. Bergquist & O. Sterner: Lachnumon and lachnumol, new metabolites with nematicidal and antimicrobial activities from the ascomycete \textit{Lachnum papracceum} (Karst.) Karst. II. Structural elucidation. J. Antibiotics 46: 968~971, 1993


8) Kalinowski, H. O.; S. Berger & S. Braun: \(^{13}C\) NMR-Spektroskopie. p. 263, Thieme Verlag, Stuttgart, 1984

