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

VI. Structure Determination of Non-halogenated Metabolites Structurally Related to Mycorrhizin A

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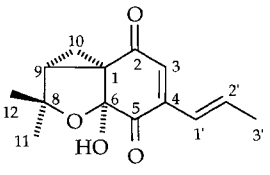
The structure determination of four new biologically active non-halogenated metabolites isolated from submerged cultures of the ascomycete *Lachnum papyraceum* is described. The compounds are structurally related to the antibiotic mycorrhizin A: (1′*Z*)-Dechloromycorrhizin A (**12**), a stereoisomer of dechloromycorrhizin A (**5**) previously isolated from the same fungus, as well as papyracon A (**13**), papyracon B (**14**) and papyracon C (**15**) containing an exocyclic double bond. The amounts of the latter three increased significantly when $CaBr_2$ was added to the culture medium. The structures were determined by spectroscopic methods.

The wood-inhabiting ascomycete *Lachnum papyraceum* is an efficient producer of chlorinated metabolites with nematicidal and antimicrobial activities in submerged cultures, producing for example lachnumon (**1**), lachnumol A (**2**), mycorrhizin A (**3**) and chloromycorrhizin A (**4**)[1,2] (the structures of all compounds **1**～**15** are given in the preceding paper, number 5 in this series). In an attempt to obtain the brominated analogues of the chlorinated metabolites, $CaBr_2$ was added to the culture medium, and this was found to strongly affect the secondary metabolism of the fungus[3,4]. When $CaBr_2$ was added at the beginning of the fermentation, the six dihydroisocoumarin derivatives **6**～**11** were formed as the major metabolites, while the production of lachnumon and mycorrhizin A derivatives instead was suppressed[3,4]. However, if $CaBr_2$ was added first at the onset of the secondary metabolism, three new non-halogenated metabolites, that in normal medium only are formed in very small amounts, were obtained together with four new brominated derivatives of lachnumon (**1**) and mycorrhizin A (**3**). In addition, (1′*Z*)-dechloromycorrhizin A (**12**), which together with its 1′*E*-isomer **5** is produced also in normal medium[1,2], was obtained in sufficient amounts for a structure determination. The isolation and biological activities of the eight new compounds are described in the preceding paper[5], while the structure determination of the non-halogenated derivatives **12**～**15** is presented in this paper.

Results and Discussion

Dechloromycorrhizin A (**5**), which previously has been prepared synthetically[6] but so far only has been isolated as a natural product from *L. papyraceum*[1], was obtained in approximately the same amounts as (1′*Z*)-dechloromycorrhizin A (**12**) and the papyracons A (**13**), B (**14**) and C (**15**)[5].



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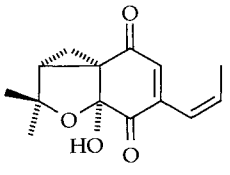

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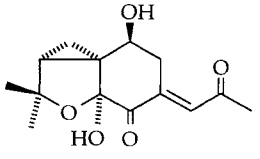

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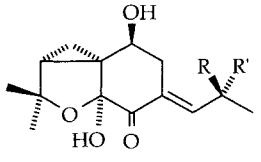


14: R=OH, R'=H
15: R=H, R'=OH

Table 1. Physico-chemical properties of compounds **12**, **13**, **14** and **15**.

	12	**13**	**14**	**15**
Appearance	Yellowish oil	Yellowish oil	Yellowish oil	Yellowish oil
$[\alpha]_D^{22}$	+49° (*c* 0.8 in acetone)	+42° (*c* 0.7 in $CHCl_3$)	+69° (*c* 1.1 in acetone)	+140° (*c* 1.3 in acetone)
Molecular formula	$C_{14}H_{16}O_4$	$C_{14}H_{18}O_5$	$C_{14}H_{20}O_5$	$C_{14}H_{20}O_5$
HREI-MS (*m/z*)				
Observed	230.0935 ($M^+ - H_2O$)	266.1136 (M^+)	250.1223 ($M^+ - H_2O$)	250.1231 ($M^+ - H_2O$)
Calculated	230.0943 for $C_{14}H_{14}O_3$	266.1154 for $C_{14}H_{18}O_5$	250.1205 for $C_{14}H_{18}O_4$	250.1205 for $C_{14}H_{18}O_4$
EI-MS	250 (5%), 248 (M^+, 2%), 230 (100%), 215 (45%), 202 (43%), 187 (60%), 159 (43%), 122 (58%), 66 (78%)	266 (2%), 251 (8%), 248 (5%), 233 (8%), 230 (16%), 205 (32%), 202 (33%), 187 (100%), 161 (44%), 139 (59%), 123 (37%), 110 (42%)	250 (13%), 235 (12%), 232 (12%), 217 (60%), 204 (24%), 189 (33%), 187 (61%), 109 (70%), 95 (66%), 43 (100%)	250 (13%), 235 (15%), 232 (11%), 217 (65%), 204 (25%), 189 (35%), 187 (66%), 109 (74%), 95 (76%), 43 (100%)
UV (MeOH)				
λ_{max} nm (ε)	218 (9,400), 300 (5,500)	251 (6,350)	245 (5,050)	246 (5,550)
IR (KBr) cm^{-1}	3400, 2980, 1710, 1670, 1630, 1390, 1310, 1175, 1100	3440, 2970, 1715, 1685, 1370, 1240, 1100, 1055, 955	3420, 2980, 1710, 1635, 1375, 1300, 1110, 1055, 965	3420, 2980, 1695, 1615, 1370, 1300, 1105, 1060, 955
TLC (Rf)	0.45[a], 0.43[b]	0.43[a], 0.42[b]	0.81[a], 0.52[b]	0.42[a], 0.52[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 of compounds **5**, **12**, **13**, **14** and **15**.

Compound proton	**5**	**12**	**13**	**14**	**15**
2-H	—	—	4.16 (dd; 3.6, 8.8)	4.08 (dd; 3.7, 8.9)	4.10 (dd; 3.8, 9.7)
3-Hα	6.78 (br s)	6.85 (br s)	3.53 (ddd; 2.1, 3.6, 17.3)	3.06 (ddd; 1.7, 3.7, 15.1)	3.11 (ddd; 1.4, 3.8, 15.2)
3-Hβ	—	—	3.16 (ddd; 2.5, 8.8, 17.3)	2.68 (ddd; 2.2, 8.9, 15.1)	2.54 (ddd; 2.3, 9.7, 15.2)
9-H	2.16 (dd; 5.6, 8.3)	2.20 (dd; 5.8, 8.3)	1.79 (dd; 4.8, 8.3)	1.74 (dd; 4.6, 8.0)	1.73 (dd; 4.5, 8.1)
10-Hα	1.57 (dd; 4.9, 5.6)	1.61 (dd; 4.9, 5.8)	1.25 (dd; 5, 5)	1.09 (dd; 4.5, 4.5)	1.04 (dd; 4.5, 4.5)
10-Hβ	1.92 (dd; 4.9, 8.3)	1.95 (dd; 4.9, 8.3)	1.02 (dd; 5.2, 8.3)	0.91 (dd; 4.5, 8.0)	0.92 (dd; 4.5, 8.1)
11-H_3	1.33 (s)	1.34 (s)	1.31 (s)	1.19 (s)	1.18 (s)
12-H_3	1.22 (s)	1.23 (s)	1.22 (s)	1.14 (s)	1.12 (s)
1′-H	6.40 (dd; 1.7, 15.9)	6.39 (ddq; 0.9, 11.9, 1.8)	7.10 (dd; 2.1, 2.5)	6.63 (ddd; 1.7, 2.2, 8.1)	6.65 (ddd; 1.4, 2.3, 8.0)
2′-H	6.73 (dq; 6.8, 15.9)	6.25 (ddq; 0.4, 11.9, 7.3)	—	4.62 (dq; 7.9, 6.5)	4.63 (dq; 8.0, 6.4)
3′-H_3	1.95 (dd; 1.7, 6.8)	1.91 (dd; 1.8, 7.3)	2.36 (s)	1.26 (d; 6.5)	1.24 (d; 6.4)
6-OH	3.20 (s)	3.15 (s)	3.39 (s)	5.15 (br s)	5.15 (br s)

The spectra were recorded in $CDCl_3$ (compounds **5**, **12** and **13**) and CD_3COCD_3 (compounds **14** and **15**) at 500 MHz. The solvent signals (7.26 and 2.05 ppm, respectively) were used as references.

The physico-chemical properties of compounds **12**, **13**, **14** and **15** are given in Table 1, while the ^1H and ^{13}C NMR data are given in Tables 2 and 3, respectively. The NMR data for dechloromycorrhizin A (**5**) have previously only been published in a thesis[7], to facilitate the comparison they are included in Tables 2 and 3.

Structure Determination of (1′*Z*)-Dechloromycorrhizin A (**12**)

The EI-MS data of (1′*Z*)-dechloromycorrhizin A (**12**) are similar to those of mycorrhizin A (**3**)[8], chloromycorrhizin A (**4**)[8] and dechloromycorrhizin A (**5**); the M+2 peak, which is typical for quinones[9] and also observed for mycorrhizin A (**5**),[8] is actually bigger than M^+ and *m/z* 230 ($M-H_2O$), 215 ($M-H_2O-CH_3$) and 202 ($M-H_2O-CO$) are important fragments. The NMR data of (1′*Z*)-dechloro-mycorrhizin A (**12**) are almost identical with those of its (1′*E*)-isomer **5** (see Tables 2 and 3), the major difference being $J_{1',2'}$ (11.9 Hz

Table 3. ^{13}C NMR data of compounds **5**, **12**, **13**, **14** and **15**.

Carbon No.	5	12	13	14	15
C-1	42.8 (s)	42.7 (s)	40.2 (s)	41.1 (s)	41.1 (s)
C-2	192.6 (s)	193.0 (s)	63.9 (d)	64.7 (d)	64.0 (d)
C-3	133.3 (d)	136.8[a] (d)	35.5 (t)	34.6 (t)	35.0 (t)
C-4	144.9 (s)	144.9 (s)	142.3 (s)	131.8 (s)	131.4 (s)
C-5	194.0 (s)	194.4 (s)	196.8 (s)	196.5 (s)	196.6 (s)
C-6	100.0 (s)	99.6 (s)	101.0 (s)	102.4 (s)	102.2 (s)
C-8	82.4 (s)	82.2 (s)	83.2 (s)	81.6 (s)	81.5 (s)
C-9	44.6 (d)	44.8 (d)	31.2 (d)	31.2 (d)	30.9 (d)
C-10	14.0 (t)	14.2 (t)	10.6 (t)	10.5 (t)	9.7 (t)
C-11	24.8 (q)	24.7 (q)	25.1 (q)	25.4 (q)	25.4 (q)
C-12	29.2 (q)	29.1 (q)	29.8 (q)	30.0 (q)	30.1 (q)
C-1′	123.7 (d)	121.7 (d)	131.8 (d)	145.9 (d)	146.7 (d)
C-2′	138.7 (d)	136.9[a] (d)	199.6 (s)	64.3 (d)	64.4 (d)
C-3′	19.6 (q)	15.8 (q)	32.2 (q)	23.1 (q)	22.7 (q)

[a] Interchangeable.

The spectra were recorded in $CDCl_3$ (compounds **5**, **12** and **13**) or CD_3COCD_3 (compounds **14** and **15**) at 500 MHz. The solvent signals (77.0 and 29.8 ppm, respectively) were used as references.

in the former and 15.9 Hz in the latter) which determines the stereochemistry of the C-1′~C-2′ double bond. The structures of both compounds were elucidated by HMQC/HMBC correlation experiments, and significant long-range correlations are displayed in Fig. 1. The same NOESY correlations were observed with compounds **5** and **12** as with mycorrhizin A (**5**), establishing the relative stereochemistry of (1′*Z*)-dechloromycorrhizin A (**12**). The absolute stereochemistry of mycorrhizin A (**3**) and chloromycorrhizin A (**4**) has been determined[8], and the same enantiomers were obtained from *L. papyraceum*.

Structure Determination of Papyracon A (13)

HREI measurements of papyracon A (**13**) indicated that its molecular composition is $C_{14}H_{18}O_5$, and this was supported by 14 signals in the ^{13}C NMR spectrum. ^1H-^{13}C correlation spectroscopy (Fig. 1) demonstrated that the carbon skeleton is the same as in the mycorrhizins, although C-2 and C-3 are reduced and C-2′ oxidised in papyracon A (**13**) compared to compound **12**. The large ^4J-allylic coupling constants between 3-H_2 and 1′-H (2.1 and 2.5 Hz) suggest it to be transoid[10], and this is supported by the missing NOESY correlation between 1′-H and 3-Hα~3-Hβ. 1′-H only gives a NOESY correlation to 3′-H_3 (see Fig. 2). NOESY correlations were also observed between 9-H as well as 12-H_3 and 3-Hβ, indicating that the latter is axial, and the magnitude of $J_{2,3\beta}$ (8.8 Hz) suggests that 2-H and 3-Hβ are trans-diaxial. This places 4-OH as shown in structure **13**, which is in accordance with the observed NOESY correlations between 2-H and 10-Hβ.

Structure Determination of Papyracon B (14) and Papyracon C (15)

The spectral data of papyracon B (**14**) and papyracon C (**15**) are very similar, suggesting that they are isomers. The NMR data (Tables 2 and 3, and Fig. 1) show that the compounds are reduced compared to papyracon A (**13**), with a C-2′ hydroxyl function instead of a keton, although the peak for M^+ (*m/z* 268) is missing in the EI mass spectra of both compounds (the heaviest ion observed is *m/z* 250, $M-H_2O$, corresponding to the composition $C_{14}H_{18}O_4$). NOESY correlations (see Fig. 2) between 12-H_3 and 3-Hβ, 12-H_3 and 9-H, 9-H and 10-Hβ, as well as 2-H and 10-Hβ in both compounds show that the relative configuration of C-1, C-2 and C-6 is the same as in papyracon A (**13**). The lack of a NOESY correlation between 1′-H and 3-Hα~3-Hβ indicates that the double bond in both compounds **14** and **15** is *E* as in papyracon A (**13**), while the different NOESY correlations between 2′-H as well as 3′-H_3 and 3-Hα~3-Hβ in papyracon B (**14**) and papyracon C (**15**) (see Fig. 2) suggest that the two compounds differ in the C-2′-configuration. In papyracon B (**14**) 2′-H gives a NOESY correlation to both 3-Hα and 3-Hβ, while 3′-H_3 only correlates to 3-Hα, and in papyracon C (**15**) 2′-H correlates to 3-Hα while 3′-H_3 correlates to both 3-Hα and 3-Hβ. These observations indicate that the C-2′~2′-OH bond is approximately parallell with the C-4~C-′1 double bond in the prefered conformation of both compounds, as displayed in Fig. 2.

Besides being members of a series of biologically and structurally interesting natural products, (1′*Z*)-dechloromycorrhizin A (**12**) and the papyracons A~C (**13**~**15**)

Fig. 1. Significant ^{1}H-^{13}C long-range correlations observed for compounds **12**~**15**.

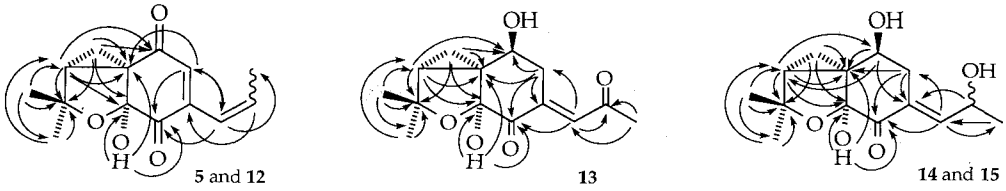
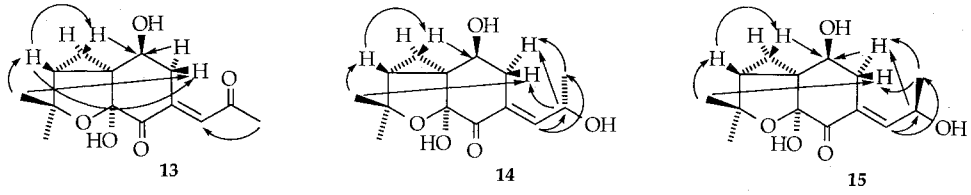


Fig. 2. NOESY correlations observed for compounds **13**~**15**.



may be important pieces in the biosynthetic puzzle of mycorrhizin A (**3**) and its derivatives. If the isocoumarins (*e.g.*, **6**) are the biogenetic precursors of the mycorrhizins, it may be significant that C-2′ in both the isocoumarins and the papyracons are oxygenated. However, the exact relationships between the different metabolites isolated from *Lachnum papyraceum* remains to be clarified.

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 $CDCl_3$ or CD_3COCD_3) were obtained with a Bruker ARX500 spectrometer. TLC experiments were performed on Merck Kieselgel 60 F_{254} precoated plates.

Acknowledgments

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