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

IV. Structural Elucidation of Novel Isocoumarin Derivatives

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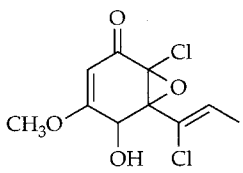
The structures of four new biologically active halogenated dihydroiso coumarins isolated from submerged cultures of the ascomycete *Lachnum papyraceum* have been elucidated by spectroscopic methods. The compounds are structurally related to lachnumon and mycorrhizin A, which are also produced by the fungus.

In a previous investigation of the nematicidal metabolites produced by submerged cultures of the wood-inhabiting ascomycete *Lachnum papyraceum*, five active metabolites were isolated[1], and characterised[2] as lachnumon (**1**), lachnumol A (**2**), mycorrhizin A (**3**), chloromycorrhizin A (**4**) and dechloromycorrhizin A (**5**). During an investigation of the influence of $CaBr_2$ on the biosynthesis of chlorinated secondary metabolites in *Lachnum papyraceum*, it was noted that the production of the mycorrhizins and lachnumon type antibiotics was strongly inhibited by the addition of 5 mM $CaBr_2$ in the culture medium[3]. Instead six dihydroisocoumarin (or isochroman-1-one) derivatives, 6,8-dihydroxy-3-methyl-isochroman-1-one (or 6-hydroxymellein[4]) (**6**), 4-chloro-6,8-dihydroxy-3-methylisochroman-1-one (**7**), 4-bromo-6,8-dihydroxy-3-methylisochroman-1-one (**9**), 8-hydroxy-6-methoxy-3-methylisochroman-1-one (or 6-methoxy-mellein[5]) (**10**), 4-chloro-8-hydroxy-6-methoxy-3-methylisochroman-1-one (**11**), and 4-chloro-5,6,8-trihydroxy-3-methylisochroman-1-one (**12**) could be isolated. While compounds **6** and **10** have been isolated from various sources and could be identified by comparing their

Structures



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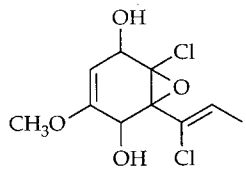

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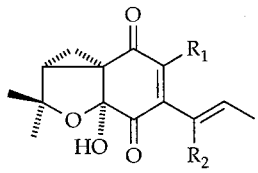
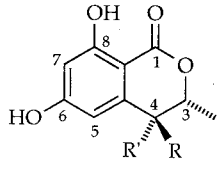
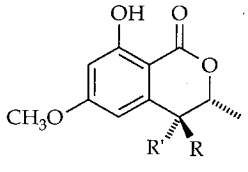


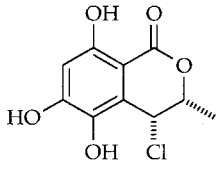
3: R_1=H, R_2=Cl
4: R_1=R_2=Cl
5: R_1=R_2=H



6: R=R'=H
7: R=H, R'=Cl
8: R=Cl, R'=H
9: R=H, R'=Br



10: R=R'=H
11: R=H, R'=Cl



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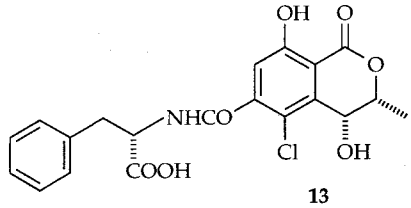

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† Dedicated to Prof. Dr. H. ZAEHNER, University of Tübingen, on the occasion of his 65th birthday.

physical data with those previously reported[4,5], compounds **7**, **9**, **11**, and **12** are new.

The structures of the dihydroisocoumarins **7**, **9**, **11** and **12** were elucidated by spectroscopic methods. The isotope patterns observed in the mass spectra of the compounds indicated that they are halogenated, and the elemental compositions given in Table 1 were suggested by high resolution mass spectroscopy. The three chlorinated compounds **7**, **11** and **12** all lose CO_2 (according to high resolution mass spectroscopy) in the EI-MS, indicating that they are carboxyl acid derivatives. The brominated compound **9** loses Br very easily, and the mass spectrum of **9** differs in that respect from those of compounds **7**, **11** and **12**. Although the solubility of several of the compounds was limited in pure chloroform, a singlet at approximately 11 ppm in the ^1H NMR spectra of the compounds recorded in $CDCl_3$ suggested that all contain a hydrogen bonded hydroxyl proton. 2D correlation NMR spectroscopy revealed the short-range ^1H-^1H and ^1H-^{13}C couplings, and the ^1H and ^{13}C NMR data for compounds **7**, **9**, **11** and **12** are given in Tables 2 and 3. In the ^1H NMR spectra of compounds **7** and **11** two meta-coupled aromatic protons can be seen (the signals for the two aromatic protons of compound **9** are overlapping), and one gives a long-range ^1H-^1H coupling with the benzylic proton. The ^{13}C chemical shifts of the corresponding benzylic carbons make this position most likely to be the one that is halogenated. Significant ^1H-^{13}C long-range correlations observed for compounds **7** and **12** are shown in Fig. 1. The corresponding correlations were also observed for compounds **9** and **11**, in addition to the correlation from 6-OCH_3 to C-6 for compound **11**.

The absolute stereochemistry of (−)-6-hydroxymellein (**6**) and (−)-6-methoxymellein (**10**) isolated in this investigation is known[6], and it is reasonable to assume that C-3 in the other dihydroisocoumarins obtained here also has the *R*-configuration. However, the enantiomers of several mellein derivatives (*e.g.* 6-hydroxymellein[7]) have also been isolated from natural sources. The assignment of the relative C-3/C-4 stereochemistry is based on comparisons with literature data. The $J_{3\text{-}4}$ of similar dihydroisocoumarins (*e.g. cis*-4-hydroxymellein[8,9] and 4-hydroxyochratoxin A **13**[10]) with the C-3 methyl and C-4 hydroxy groups *cis* are approximately 2 Hz, while $J_{3\text{-}4}$ in *trans*-4-hydroxymellein, isolated from *Apiospora camptospora*, has been reported to be 4 Hz[8]. Both isomers of 4-hydroxy-5-methylmellein were isolated as phytotoxic metabolites of the fungus *Valsa ceratosperma*[11], and the $J_{3\text{-}4}$ is unexpectedly reported to be

Fig. 1. Significant ^1H-^{13}C long-range correlations observed with compounds **7** and **12**.

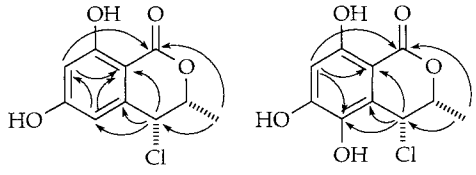

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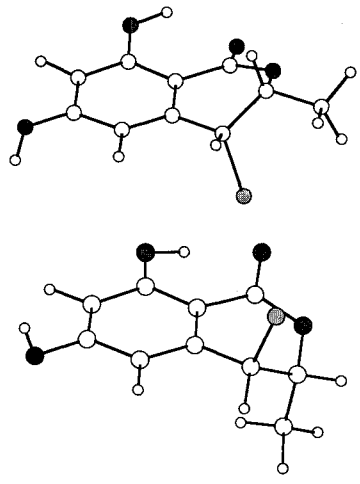
Fig. 2. The most stable conformations of compound **7** (top) and compound **8** (bottom). See also Table 4.



1.5 Hz for the *cis* isomer and 1.4 Hz for the *trans* isomer. A larger difference was on the other hand noted for the ^1H chemical shift of the C-3 methyl groups of the two isomers, which is 1.63 ppm for the *cis* isomer and 1.28 ppm for the *trans* isomer (while it is 1.48 ppm for 6-hydroxymellein (**6**)). The values can be explained if the two isomers exist in different conformations[11], with the C-3 methyl group in an axial position and thereby shielded by the benzene ring in the *trans* isomer. Molecular mechanics calculations with the isomers **7** and **8** (compound **8** is hypothetical) confirm this (see the experimental part and Table 4), and the most stable conformations of compounds **7** and **8** are shown in Fig. 2. (The identical calculations with compound **12** and its hypothetical trans isomer gave very similar results, data not shown.) The $J_{3\text{-}4}$ of the C-4 halogenated derivatives **7**, **9**, **11** and **12** are all close to 2 Hz (see Table 2) and the chemical shift for the C-3 methyl groups are between 1.5 and 1.6 ppm, suggesting that they all are *cis* isomers.

3-Methyldihydroisocoumarins with aromatic carbons chlorinated instead of C-4 have previously been isolated from *Sporormia affinis* and *Periconia macrospinosa*[4,12]. It has also been shown that such dihydroisocoumarins are polyketide metabolites and that they are precursors to the chlorinated cyclopentenes in *Periconia macro-*

Table 1. Physico-chemical properties of compounds **7**, **9**, **11** and **12**.

	7	**9**	**11**	**12**
Appearance	Colourless crystals	Colourless crystals	Colourless crystals	Colourless crystals
MP (°C)	165~169	179~183	131~133	120~123
$[\alpha]_D^{22}$	−75° (*c* 1.0 in $CHCl_3$)	−145° (*c* 2.0 in $CHCl_3$)	−69° (*c* 0.1 in $CHCl_3$)	−5.7° (*c* 0.4 in $CHCl_3$)
Molecular formula	$C_{10}H_9O_4Cl$	$C_{10}H_9O_4Br$	$C_{11}H_{11}O_4Cl$	$C_{10}H_9O_5Cl$
HREI-MS (*m/z*)				
Observed	228.0173 M^+	271.9697 M^+	242.0353 M^+	244.0137 M^+
Calculated	228.0189 for $C_{10}H_9O_4{}^{35}Cl$	271.9685 for $C_{10}H_9O_4{}^{79}Br$	242.0346 for $C_{11}H_{11}O_4{}^{35}Cl$	244.0138 for $C_{10}H_9O_5{}^{35}Cl$
EI-MS	230 (35% of 228), 228 (61%), 193 (10%), 186 (35% of 184), 184 (100%), 165 (72%), 150 (13%), 121 (22%)	274 (100% of 272), 272 (18%), 193 (100%), 165 (68%), 150 (22%), 121 (23%)	244 (35% of 242), 242 (75%), 207 (16%), 200 (35% of 198), 198 (100%), 179 (70%), 164 (12%), 135 (19%)	246 (35% of 244), 244 (88%), 217 (35% of 215), 215 (19%), 202 (35% of 200), 200 (57%), 171 (100%)
UV (MeOH) λ_{max} nm (ε)	220 (15,700), 269 (7,600), 309 (4,700)	231 (6,200), 272 (2,900), 313 (2,000)	219 (7,800), 268 (3,500), 308 (2,100)	219 (5,400), 268 (2,200), 314 (1,600)
IR (KBr) cm^{-1}	3420, 3190, 1665, 1630, 1385, 1250, 1165, 1105	3420, 3200, 1665, 1630, 1380, 1250, 1165, 1110	3440, 1675, 1620, 1315, 1265, 1195, 1175, 1110	3400, 1660, 1610, 1380, 1240, 1120
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 F_{254}: Toluene - aceton - AcOH (70 : 30 : 1).
[b] Merck, Kieselgel 60 F_{254}: Toluene - ethyl formiat - formic acid (10 : 5 : 3).

Table 2. ^1H NMR data of compounds **7**, **9**, **11**, and **12**. The spectra were recorded in $CDCl_3$ (compound **11**) or $CDCl_3$: CD_3OD 20 : 1 at 500 MHz. The $CDCl_3$ signal (7.26 ppm) was used as a reference.

Proton:	**7**	**9**	**11**	**12**
3-H	4.75 (dq; 2.0, 6.3)	4.42 (dq; 2.0, 6.3)	4.79 (dq; 2.0, 6.4)	4.52 (dq; 1.9, 6.6)
4-H	4.80 (dd; 0.5, 2.0)	4.94 (dd; 0.3, 2.0)	4.84 (dd; 0.5, 2.0)	4.78 (d; 1.9)
5-H	6.36 (dd; 0.5, 2.3)	6.31 (m)	6.45 (dd; 0.5, 2.3)	—
7-H	6.37 (d; 2.3)	6.31 (m)	6.48 (d; 2.3)	6.49 (s)
9-H_3	1.57 (d; 6.3)	1.52 (d; 6.3)	1.61 (d; 6.4)	1.55 (d; 6.6)
6-OCH_3	—	—	3.84 (s)	—
8-OH	—	—	11.12 (s)	—

Table 3. ^{13}C NMR data of compounds **7**, **9**, **11**, and **12**. The spectra were recorded in $CDCl_3$ (compound **11**) or $CDCl_3$: CD_3OD 20 : 1 at 125 MHz, and the $CDCl_3$ signal (77.0 ppm) was used as a reference.

Carbon No.	**7**	**9**	**11**	**12**
C-1	168.6 (s)	168.7 (s)	168.4 (s)	168.6 (s)
C-3	76.0 (d)	75.8 (d)	76.0 (d)	77.5 (d)
C-4	57.0 (d)	49.2 (d)	56.9 (d)	64.6 (d)
C-4a	141.2 (s)	142.4 (s)	140.8 (s)	138.6 (s)
C-5	107.4 (d)	107.1 (d)	106.8 (d)	110.2 (s)
C-6	164.2[a] (s)	164.6[a] (s)	166.0 (s)	163.0[a] (s)
C-7	103.8 (d)	103.7 (d)	101.7 (d)	104.8 (d)
C-8	164.5[a] (s)	164.1[a] (s)	164.8 (s)	158.2[a] (s)
C-8a	97.5 (s)	98.9 (s)	99.6 (s)	101.7 (s)
C-9	17.8 (q)	19.6 (q)	17.9 (q)	16.1 (q)
OCH_3			55.8 (q)	

[a] Interchangable.

Table 4. The steric energies (in kcal/mol) of the two most stable conformers of compound **7** and **8**, obtained by molecular mechanics calculations. See also Fig. 2.

Compound	C-3 methyl:	Axial	Equatorial
7		1.98	4.54
8		4.20	1.70

spinosa[13]. The co-isolation of the dihydroisocoumarins with the mycorrhizins (compounds **3**~**5**) supports the suggestion[14] that the the biosynthesis of the latter preceeds *via* dihydroisocoumarins.

Experimental

The compounds were isolated from the organic extract of a culture filtrate of the fungus *Lachnum papyraceum*[3]. 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 $CDCl_3$ - CD_3OD, 20 : 1) were obtained with a Bruker ARX500 spectrometer. 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. The MM calculations were made with the MacMimic program (version 2.9), obtained from InStar Software AB (Lund, Sweden), on a Macintosh Quadra 700. The torsion parameters for C (carbonyl)–C(sp 2)–C(sp 2)–O(sp 3) were not provided in the program, and were set to: V1 = 0.0; V2 = 15.0; V3 = 0.0.

Acknowledgments

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