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Novel monochlorinated metabolites with a 1-benzoxepin skeleton from *Mycena galopus*

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Abstract

Two novel monochlorinated 2,3-dihydro-1-benzoxepin derivatives were isolated from the ethyl acetate extracts of the stipes of *Mycena galopus*. The structures were established on the basis of their spectral data.

Keywords: natural products; fungi; benzoxepins.

Basidiomycetes as decomposers of forest litter represent an ecologically important group of organisms in the environment and are known to produce a wide variety of organohalogen compounds.[1] An important group of organohalogen producers are the fungal species belonging to the genera *Bjerkandera*, *Hypholoma* and *Mycena*.[2] Fungi belonging to the genus *Mycena*, for instance, produce in addition to the commonly occurring chlorinated *p*-anisyl metabolites[3] and chlorinated hydroquinone methyl ethers,[4] several other chlorinated metabolites such as chloroform,[5] tetrachlorocatechol and its methyl ether[6] and the bioactive compounds strobilurin B[7] and mycenon.[8] In a recent study probe-MS indicated the presence of a monochlorinated component in the latex of *Mycena galopus* (Pers.: Fr.) Kummer, but the identity of this unknown compound could not be established.[9] Therefore, as part of our continuing search for novel halometabolites from basidiomycetes, we decided to reinvestigate the latex of *M. galopus* in more detail. In the present paper, we describe the isolation and structural elucidation of two novel chlorinated constituents (**1** and **2**) from *M. galopus* which were not previously reported as metabolites from basidiomycetes or any other living organism.

M. galopus is a common saprotrophic agaric in angiospermous and coniferous woodlands of northern temperate regions of the world and, when fresh, is readily identified by the white latex that can be

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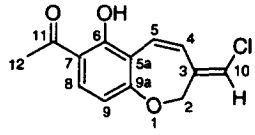
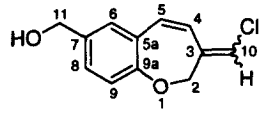
squeezed from the stipe. The fruit bodies were collected in the Grizedale Forest (Cumbria, United Kingdom) between September 24th and November 4th, 1998. Care was taken to collect only fresh specimens since the diagnostic latex is often absent after frost or when the fruit bodies are ageing.

The pilei were cut off and the stipes cleaned of adhering litter. The stipes were frozen with liquid N_2, pulverized and freeze-dried to afford a fine brown powder (2.28 g). Extraction of the powder with EtOAc gave a yellow oil (0.314 g) which was fractionated over a silica gel column using gradient elution with pentane:*tert*-butyl methyl ether (100:0 → 3:2). GC–MS was used to examine the fractions for the presence of Cl in the eluted compounds. This procedure led to the isolation of 0.3 mg of **1**[†] (purity >98%) and 0.3 mg of **2**[‡] as an inseparable 3:1 mixture of *E* and *Z* isomers, respectively. In spite of these very small amounts, the structures of **1** and **2** could be established using 1D NMR spectroscopy in combination with 2D NOESY and spin-decoupling experiments.



1



2 (*E* and *Z*)

The less polar compound **1** was obtained as a light yellow solid (mp 138–141°C), whose molecular formula was established as $C_{13}H_{11}ClO_3$ by HREIMS. The 3:1 ratio of $[M]^+$ and $[M+2]^+$ at *m/z* 250 and 252, respectively, observed in the EIMS confirmed the presence of one Cl atom in **1**. The ^{13}C NMR and DEPT spectra revealed the presence of one CH_3, one oxygenated CH_2 (δ 73.6), five CH and six quarternary carbons, including a carbonyl carbon (δ 203.6) and two oxygen-bearing aromatic carbons (δ 164.2 and 166.3) *meta*-situated to each other. From the ^{13}C NMR data, it was obvious that **1** should possess an ether linkage (Ar-O-CH_2-) and an aromatic OH group. The ^1H NMR spectrum of **1** (Table 1) exhibited two *ortho*-coupled (J=8.9 Hz) aromatic proton signals at δ 7.57 and 6.52 indicative of a 1,2,3,4-tetrasubstituted benzene system, a two-proton signal at δ 4.61 confirming the presence of the ether linkage, a Me singlet at δ 2.58 and a sharp singlet at δ 13.54 (exchangeable with CD_3OD) due to a chelated OH function. The latter two signals, together with a prominent fragment ion peak at *m/z* 43 in the EIMS and the carbonyl carbon resonance at δ 203.6, pointed to the presence of an acetyl group adjacent to a phenolic OH. The three remaining one-proton signals at δ 7.14, 6.87 and 6.10 suggested the presence of a di- and trisubstituted double bond in the molecule. Since the molecular formula $C_{13}H_{11}ClO_3$ required eight degrees of unsaturation, compound **1** should have one additional ring system apart from the above groups which collectively accounted for seven degrees of unsaturation. Spin-decoupling experiments and measurement of coupling constants enabled signals in the ^1H NMR spectrum of **1** to be correlated and led directly to the conjugated diene fragment C(5)H=C(4)H-C(3)=C(10)H(Cl). Decoupling of H-5 removed the small splitting (0.5 Hz) of H-9 and suggested the insertion of two quarternary aromatic carbons,

[†] (*E*)-**1**: ^1H NMR see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9 (q), 73.6 (t), 111.6 (d), 115.4 (s), 116.4 (s), 119.6 (d), 123.2 (d), 124.1 (d), 131.6 (d), 136.4 (s), 164.2 (s), 166.3 (s), 203.6 (s); EIMS *m/z* (rel. int.%) 252 (M^++2, 33), 250 (M^+, 100), 235 (14), 215 (41), 197 (13), 173 (24), 161 (41), 137 (10), 115 (31), 105 (26), 95 (9), 77 (13), 63 (8), 51 (9), 43 (38); HREIMS calcd for $C_{13}H_{11}ClO_3$: 250.0397. Found: 250.0395.

[‡] (*E*)-**2**: ^1H NMR see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.1, 73.0, 119.7, 120.6, 124.5, 127.4, 128.6, 131.5, 132.4, 135.9, 136.8, 159.2; EIMS *m/z* (rel. int.%) 224 (M^++2, 28), 222 (M^+, 85), 187 (100), 169 (24), 157 (31), 141 (67), 129 (54), 128 (78), 127 (39), 115 (37), 91 (36), 77 (23), 63 (18), 51 (21), 39 (17); HREIMS calcd for $C_{12}H_{11}ClO_2$: 222.0448. Found: 222.0445. (*Z*)-**2**: ^1H NMR see Table 1; EIMS *m/z* (rel. int.%) 224 (M^++2, 29), 222 (M^+, 87), 187 (100), 169 (25), 157 (31), 141 (69), 129 (58), 128 (84), 127 (42), 115 (38), 91 (38), 77 (23), 63 (18), 51 (21), 39 (17); HREIMS calcd for $C_{12}H_{11}ClO_2$: 222.0448. Found: 222.0446.

Table 1
^{1}H NMR spectral data (δ values, *J* Hz in parentheses) for **1**, (*E*)-**2** and (*Z*)-**2**

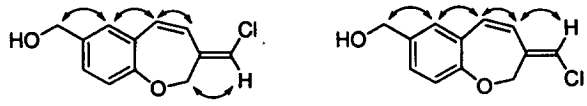
C-number	**1**[a]	(*E*)-**2**[b]	(*Z*)-**2**[b]
2	4.61, 2H, dd (0.8, 0.5)	4.09, 2H, br s	4.69, 2H, s
4	6.87, 1H, ddt (12.2, 1.1, 0.5)	6.80, 1H, br d (11.8)	5.83, 1H, d (11.8)
5	7.14, 1H, ddd (12.2, 1.5, 0.5)	6.24, 1H, dd (11.8, 1.4)	6.06, 1H, br d (11.8)
6	–	6.93, 1H, d (2.2)	6.96[c] (obscured)
8	7.57, 1H, d (8.9)	6.89, 1H, dd (8.2, 2.2)	6.87, 1H, dd (8.2, 2.2)
9	6.52, 1H, dd (8.9, 0.5)	6.97, 1H, d (8.2)	6.97, 1H, d (8.2)
10	6.10, 1H, ddt (1.5, 1.1, 0.8)	5.39, 1H, m	5.71, 1H, br s
11	–	4.20, 2H, s	4.22, 2H, s
12	2.58, 3H, s	–	–
6-OH	13.54, 1H, s	–	–
11-OH	–	1.30, 1H, br s	1.30, 1H, br s

[a] Measured at 400 MHz in $CDCl_3$.
[b] Measured at 500 MHz in C_6D_6.
[c] Estimated value from NOESY experiment.

C(5a) and C(9a), between H-5 and H-9. Furthermore, decoupling of H-4 and H-10 removed the small splittings (0.5 and 0.8 Hz, respectively), of the C(2) two-proton signal at δ 4.61 and suggested connection of C(2) with C(3). On the basis of these data, the structure of **1** was formulated as 1-[3-chloromethylene)-2,3-dihydro-6-hydroxy-1-benzoxepin-7-yl]-ethanone. The long-range coupling (^5J=1.5 Hz) of H-5 with H-10 pointed to the *E* configuration of the exocyclic double bond in **1**.[10] A NOE difference experiment in which irradiation of H-10 resulted in a strong positive enhancement of the C-2 two-proton signal confirmed this assignment.

GC–MS analysis of the more polar metabolite **2** (one spot on TLC with several solvents) revealed a 3:1 mixture of two isomeric compounds with almost identical EIMS. The presence of one Cl in both constituents of the mixture was concluded from the 3:1 ratio of $[M]^+$ and $[M+2]^+$ at *m/z* 222 and 224, respectively, and HREIMS measurements which established the molecular formulae as $C_{12}H_{11}ClO_2$. The ^{13}C NMR spectrum of **2** showed twelve distinct peaks which were attributed to the major isomer. The amount of the minor isomer in the sample was insufficient for ^{13}C NMR. The resonances appearing at δ 159.2, 73.0 and 65.1 suggested the presence of one oxygenated aromatic carbon and two oxygen-bearing nonaromatic carbons. The ^{1}H NMR spectrum (C_6D_6) of **2** (Table 1), in which almost all signals were resolved, suggested both isomers to have a similar 1-benzoxepin framework as found for **1**, but without the OH group at C(6) and a different substituent at C(7). The ^{1}H NMR data attributed to the major isomer indicated the presence of a 1,3,4-trisubstituted aromatic ring as followed from the three mutually coupled aromatic proton signals at δ 6.93 (d, *J*=2.2 Hz), 6.89 (dd, *J*=8.2, 2.2 Hz) and 6.97 (d, *J*=8.2 Hz). The appearance of a two-proton singlet at δ 4.20 pointed to the presence of a hydroxylated CH_2 group at C(7) which was supported by the carbon signal at δ 65.1. The splitting patterns of the aromatic protons and the two-proton singlet at δ 4.22 observed for the minor isomer suggested an identical 1,3,4-trisubstituted aromatic ring system as present in the major isomer. From these data, the chemical shifts and coupling constants of the remaining proton signals, it was concluded that **2** (1-[3-chloromethylene)-2,3-dihydro-1-benzoxepin-7-yl]-methanol) consisted of two geometric isomers (*E*)-**2** and (*Z*)-**2**, with (*E*)-**2** as the major isomer. The 2D NOESY spectrum of **2** in which all key NOE correlations expected for structures (*E*)-**2** and (*Z*)-**2** were observed, gave further support to this conclusion (Fig. 1).

Compounds **1** and **2** have not been previously described in the literature, but are structurally related to pterulone, pterulinic acid and pterulone B, recently isolated from fermentations of a *Pterula* species.[11,12] These latter compounds were found to possess antibiotic activities,[12,13] and we believe that **1** and **2**



(*E*)-**2** (*Z*)-**2**

Figure 1. Key NOESY correlations observed with (*E*)-**2** and (*Z*)-**2**

will show similar activities as previous studies have revealed antibiotic activity of culture filtrates of *M. galopus*.[14,15] Since the amounts of natural **1** and **2** were too small for bioactivity tests, a synthetic study toward these compounds is currently ongoing in our laboratory.

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