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Benzoxepine Esters as Precursors of the Wound-Activated Chemical Defence of *Mycena galopus*

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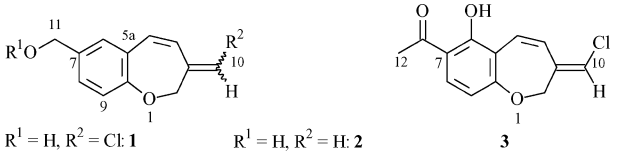
The native precursors ***E/Z*-1a** to ***E/Z*-1e** and **2a**–**2e** of the antifungally active benzoxepines ***E/Z*-1** and **2** have been isolated from intact fruiting bodies of *Mycena galopus*. These precursors turned out to be fatty acid esters of the benzoxepines (pterulones) ***E/Z*-1** and **2**. Metabolic profiling of ethyl acetate extracts from intact fruiting bodies, from the latex and from mechanically wounded fruiting bodies revealed that the known antifungally active benzoxepines ***E/Z*-1** and the previously unknown benzoxepines **2** are only present in injured fruiting bodies. Intact fruiting bodies and the latex, however, exclusively contain the corresponding ester derivatives ***E/Z*-1a** to ***E/Z*-1e** and **2a**–**2e**. Unlike the antifungally active benzoxepines ***E/Z*-1** and **2**, the esters ***E/Z*-1a** to ***E/Z*-1e** present in intact fruiting bodies turned out to be inactive. Upon injury the inactive esters ***E/Z*-1a** to ***E/Z*-1e** are cleaved by a putative esterase, and the bioactive benzoxepines ***E/Z*-1** and **2** are released, suggesting a wound-activated defence mechanism of *M. galopus* against yeasts and parasitic fungi.

Introduction

The basidiomycete *Mycena galopus* (Pers.: Fr.) P. Kumm. (German name: Weißmilchender Helmling) is a small mushroom widespread in Europe and Northern America on mossy floors of coniferous forests.[1] The fruiting bodies of *M. galopus* can easily be identified, as they contain a characteristic white latex that is exuded if they are cut or bruised. Recently, we isolated red pyrroloquinoline alkaloids from the red latex of the related species *Mycena sanguinolenta* and *Mycena haematopus* and from the red fruiting bodies of *Mycena rosea*.[2] The broad spectrum of unusual natural products detected in *Mycena* species inspired us to extend these investigations to *M. galopus*. This *Mycena* species had already been screened for the presence of bioactive compounds in 1999 by Wijnberg et al., who identified the antifungal benzoxepines (pterulones) ***E/Z*-1** and **3**.[3] 6-Hydroxypterulone (**3**) is structurally closely related to pterulone, which was isolated by Anke et al. from submerged cultures of the *Pterula* species 82168 in 1997.[4] The antifungal activity of pterulone is based on selective inhibition of the NADH:ubiquinone oxidoreductase (complex I) of the respiratory chain.[4b]

In this paper we describe the isolation and the structural elucidation of ***E/Z*-1a** to ***E/Z*-1e** and **2a**–**2e**, native constituents of the white latex, which turned out to be fatty acid esters of ***E/Z*-1** and of the previously unknown non-chlorinated benzoxepine **2**, respectively (Figure 1). Moreover, we propose that the fatty acid esters of ***E/Z*-1** serve as stable precursors of the bioactive benzoxepine alcohols ***E/Z*-1** and **2** in the wound-activated chemical defence of *M. galopus*.



R^1 = H, R^2 = Cl: **1**	R^1 = H, R^2 = H: **2**	**3**
R^1 = palmityl, R^2 = Cl: **1a**	R^1 = palmityl, R^2 = H: **2a**	
R^1 = palmitoleyl, R^2 = Cl: **1b**	R^1 = palmitoleyl, R^2 = H: **2b**	
R^1 = stearyl, R^2 = Cl: **1c**	R^1 = stearyl, R^2 = H: **2c**	
R^1 = oleyl, R^2 = Cl: **1d**	R^1 = oleyl, R^2 = H: **2d**	
R^1 = linoleyl, R^2 = Cl: **1e**	R^1 = linoleyl, R^2 = H: **2e**	

Figure 1. Benzoxepines from *Mycena galopus*.

Results and Discussion

When we reinvestigated intact fruiting bodies of *M. galopus* for the presence of the benzoxepines ***E/Z*-1** and **3**, we found ***E/Z*-1** either only in traces or not at all. A comparison of the metabolic profiles of intact fruiting bodies with those of mechanically injured ones recorded by GC-MS indicated that ***E/Z*-1** was only present in injured fruiting bodies. However, in the GC-MS of intact fruiting bodies a characteristic pair of the fragment ions *m/z* = 205/207 (which is also typical of the trimethylsilyl ethers of the benzoxepines ***E/Z*-1**) was recognised at later retention times than determined for ***E/Z*-1**, pointing to the presence of derivatives of ***E/Z*-1** in the latex instead.

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In order to isolate the new compounds from the fruiting bodies of *M. galopus*, the fungal material was frozen with liquid nitrogen immediately after collection and stored at –35 °C to avoid decomposition. The native latex constituents ***E*/*Z*-1a** to ***E*/*Z*-1e** and **2a**–**2e** were extracted with ethyl acetate from frozen fruiting bodies of *M. galopus* and separated first by preparative HPLC on RP-18 and after that on silica gel. In this way the esters ***E*/*Z*-1a** to ***E*/*Z*-1e** and **2a**–**2e** were separated from each other. A sample of frozen fruiting bodies (100 g) yielded ***E*-1a** (5.7 mg), ***Z*-1a** (1.8 mg), ***E*-1b** (1.0 mg), ***Z*-1b** (0.5 mg), ***E*-1d** (2.5 mg), ***E*-1e** (2.0 mg), ***Z*-1e** (0.7 mg), **2a** (1.7 mg) and **2e** (0.7 mg), while the trace compounds ***E*-1c**, ***Z*-1c**, ***Z*-1d**, **2b**, **2c**, **2d** and **3** were only identified by GC-MS.

The mass spectrum of the benzoxepine ***E*-1a** exhibited the same pair of base ions at *m*/*z* (^{35}Cl) = 205 and *m*/*z* (^{37}Cl) = 207 as the trimethylsilyl derivative of ***E*-1**. In addition, ***E*-1a** showed fragment ions at *m*/*z* = 170, 141, 131, 128 and 115 with intensities similar to those of the trimethylsilyl derivative of ***E*-1**, indicating that the two compounds are likely to contain the same benzoxepine moiety. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data for ***E*-1** with those for ***E*-1a** confirms this conclusion, since for all resonances present in ***E*-1** a corresponding one is present in ***E*-1a** (Table 1).

Moreover, the COSY, HSQC, HMBC and NOESY correlations allowed the complete assignment of the protons and carbon atoms of the benzoxepine fragment (Table 1 and Figure 2). However, ***E*-1a** exhibited additional signals at δ_H = 0.88, 1.24–1.25, 1.28, 1.29, 1.63, 2.34 ppm, typical of the presence of a saturated straight-chain fatty acid moiety. In the HMBC spectrum a ^{3}J correlation of the signal of the benzylic protons at δ_H = 5.05 ppm to the carbon signal at δ_C = 173.7 ppm indicates that the fatty acid moiety is esterified with the benzylic alcohol of the benzoxepine moiety (Figure 2). If the molecular mass ions at *m*/*z* = 460 [M(^{35}Cl)]$^+$ and 462 [M(^{37}Cl)]$^+$ are taken into account, ***E*-1a** consists of palmitic acid esterified with the benzoxepine alcohol ***E*-1**. To confirm this deduction, the benzoxepine ester was saponified with a methanolic solution (0.02 M) of potassium hydroxide. After trimethylsilylation with MSTFA, the mixture was analysed by GC-MS. The palmitic acid and ***E*-1** present in the hydrolysate of ***E*-1a** were identified unambiguously by GC-MS comparison of the trimethylsilylated hydrolysate with authentic samples of trimethylsilyl palmitate and the trimethylsilylated benzoxepine alcohol ***E*-1**.

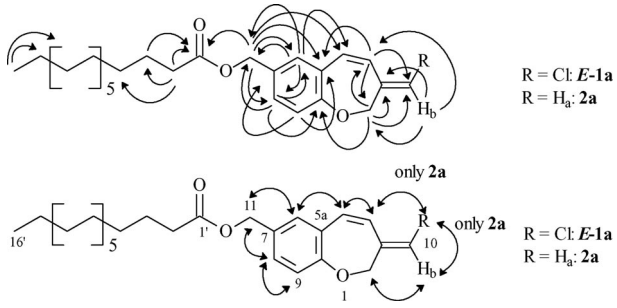

Figure 2. Selected HMBC (→) and NOE (↔) correlations of ***E*-1a** and **2a**.

The benzoxepine ester ***Z*-1a** exhibited essentially the same MS characteristics (EI) as ***E*-1a**. However, its retention time on the GC column was slightly shorter than that of ***E*-1a**. Hydrolysis of the ester ***Z*-1a** yielded ***Z*-1** and palmitic acid according to the GC-MS analysis.

The non-chlorinated ester **2a** differs from ***E*/*Z*-1a** in its GC-MS, exhibiting a molecular ion peak at *m*/*z* = 426 [M]$^+$ and a base ion peak at *m*/*z* = 171, indicating the absence of chlorine atoms. In addition, its retention time in

Table 1. NMR spectroscopic data (600 MHz, $CDCl_3$, 298 K) for ***E*-1a**, ***E*-1** and **2a**.

#	***E*-1a**		***E*-1**	**2a**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_C	δ_H (mult. *J* in Hz)
2	72.6	4.55 (s)	72.6	74.6	4.58 (s)
3	136.3		136.3	143.6	
4	124.2	6.82 (d, 11.8)	124.2	131.0	6.47 (d, 11.5)
5	130.9	6.53 (d, 11.8)	130.9	128.0	6.34 (d, 11.5)
5a	130.8		130.8	130.7	
6	133.4	7.26 (d, 1.9)	133.4	132.8	7.21 (s)
7	127.0		127.0	128.8	
8	129.4	7.18 (dd, 8.2, 1.9)	129.5	128.4	7.15 (d, 8.7)
9	120.3	6.98 (d, 8.2)	120.3	120.3	6.98 (d, 8.7)
9a	159.2		159.2	159.4	
10	119.4	6.12 (s)	119.4	117.7	5.07 (s) H_b 5.24 (s) H_a
11	65.4	5.05 (s)	65.4	65.6	5.04 (s)
1′	173.7			173.8	
2′	34.3	2.34 (t, 7.5)		34.3	2.33 (t, 7.0)
3′	24.9	1.63 (tt, 7.5, 7.5)		25.0	1.63 (tt, 7.0, 7.0)
4′	29.1	1.29 (m)		29.1	
5′–13′	29.3–29.7	1.24–1.25 (m)		29.3 to 29.7	1.24–1.25 (m)
14′	31.9	1.25 (m)		31.9	1.25 (m)
15′	23.0	1.28 (m)		22.7	1.28 (m)
16′	14.1	0.88 (t, 7.3)		14.1	0.88 (t, 7.6)

the GC-MS was considerably shorter than that of ***E*-1a** (Figure 4). In the ^{1}H NMR spectrum the signals of two protons of the exocyclic methylene group at δ_H = 5.07 and 5.24 ppm are present in **2a** instead of only one in ***E*-1a**. The protons of the exocyclic methylene group exhibit characteristic NOEs to 2-H and 4-H (Figure 2 and Table 1), thus allowing the unambiguous assignment of these protons in **2a**. In addition, hydrolysis of **2a** yielded the free benzoxepine alcohol **2** and palmitic acid as verified by GC-MS analysis.

The structures of ***E*/*Z*-1b** to ***E*/*Z*-1e** and of **2b**–**2e** were determined analogously to those of ***E*/*Z*-1a** and **2a**, respectively. In general, the GC-MS retention times of the (*Z*) isomers turned out to be slightly shorter than those of the corresponding (*E*) isomers.

In order to elucidate the ecological roles of the free alcohols ***E*/*Z*-1** and **2** and of the benzoxepine esters ***E*/*Z*-1a** to ***E*/*Z*-1e** and **2a**–**2e**, the bioactivities of the compounds were evaluated. None of the compounds exhibited any significant bioactivity towards bacteria such as *Escherichia coli*, *Bacillus brevis* or *Bacillus subtilis*. In contrast, ***E*-1** and a 1:1 mixture of ***E*/*Z*-1** exhibited moderate activities against the fungus *Cladosporium cucumerinum* and, as already demonstrated by Wijnberg,[3b] significant activities against yeasts such as *Saccharomyces cerevisiae* (Figure 3). The non-chlorinated alcohol **2** turned out to be less active towards *S. cerevisiae* than ***E*-1** or ***E*/*Z*-1** (Figure 3). The presence of the benzoxepine moiety seems to be important for the bioactivity, since benzyl alcohol itself was completely inactive. Similarly, when we tested the benzoxepine esters ***E*/*Z*-1a** to ***E*/*Z*-1e** and **2a**–**2e** against *C. cucumerinum* and *S. cerevisiae*, no growth inhibition occurred, demonstrating that the benzoxepine esters were inactive (Figure 3).

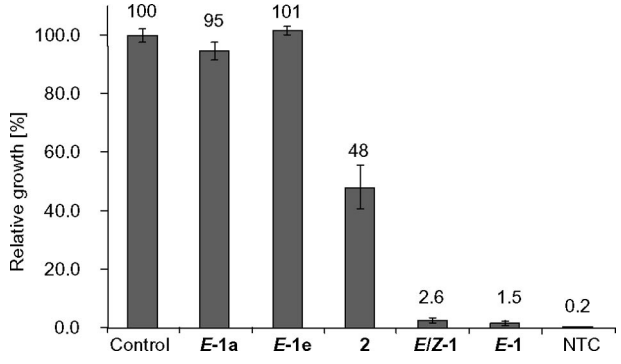

Figure 3. Bioactivities of the esters ***E*-1a** and ***E*-1e**, the alcohols ***E*/*Z*-1** (1:1 mixture), ***E*-1** and **2** and of the antibiotic nourseothricin (NTC) towards *S. cerevisiae* in YMG medium at a concentration of 1.0 mM.

If intact fruiting bodies of *M. galopus* were submerged in methanol only a few seconds after collection, the free benzoxepine alcohols ***E*/*Z*-1** and **2** were not detectable at all by GC-MS analysis of this methanolic extract (Figure 4). In contrast, after artificial injury of intact fruiting bodies by bruising, ***E*/*Z*-1** and **2** were generated. The course of the reaction was monitored by transferring injured fruiting bodies into methanol after certain periods of time to stop the enzymatic hydrolysis. The samples were analysed by GC-MS after trimethylsilylation. After 60 min, approximately the same amounts of free alcohols as of the corresponding esters were present in the extract. After 180 min, two thirds of the esters had been converted into the benzoxepine alcohols ***E*/*Z*-1** and **2**, and after 24 h, the esters had been cleaved quantitatively into the corresponding alcohols (Figure 4).

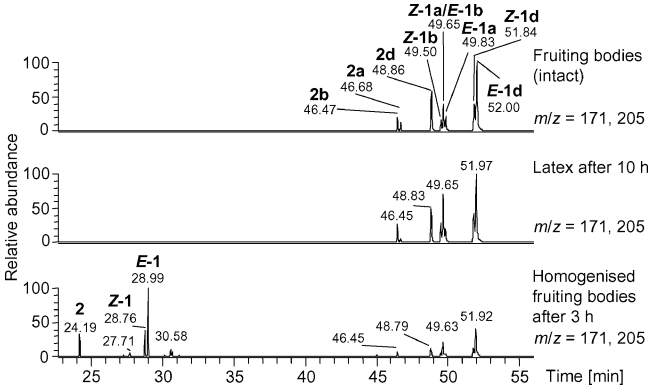

Figure 4. GC of trimethylsilylated methanolic extracts from intact fruiting bodies (top), from the latex (centre) and from homogenised fruiting bodies (bottom) of *M. galopus*.

The white latex dripping from the stipes of fresh fruiting bodies only consisted – according to the GC-MS analysis – of the benzoxepine esters together with some free fatty acids. Interestingly, even after 10 h, the latex had not decomposed at all to give the free alcohols ***E*/*Z*-1** and **2** (Figure 4). Hence, the latex itself does not contain esterases.

We therefore propose that upon wounding of the fruiting bodies, esterases come into contact with the latex and cleave the benzoxepine esters to release the corresponding bioactive alcohols ***E*/*Z*-1** and **2**. It is likely that the effects of mechanical wounding of *M. galopus* are comparable to those of injury caused to the fruiting bodies by insect feeding and infection with parasites. This assumption is supported by recent experiments with lima beans (*Phaseolus lunatus*). Boland et al. demonstrated for this example that continuous mechanical wounding induces defence reactions similar to those towards insect feeding, indicating that wounding is a crucial element of defence activation.[5]

In agreement with the proposed wound-activated chemical defence mechanism, the fruiting bodies of *M. galopus* are rarely attacked by fungivores and parasitic fungi. However, *M. galopus* is prone to infection with the mycoparasitic zygomycete *Spinellus fusiger*, which is able to infect many *Mycena* species, especially in wet and cold weather. *S. fusiger* indeed turned out to be insensitive to ***E*/*Z*-1**, thus enabling the mycoparasite to grow on fruiting bodies of *M. galopus*.

The putative esterase of *M. galopus* exhibits a broad substrate specificity. Incubation experiments with homogenised fungal material of *M. galopus* with selected esters showed that the enzymes present in *M. galopus* are able to cleave

methyl oleate and the acetylated and caprylated derivatives of the benzoxepine alcohols ***E/Z*-1** quantitatively. Even benzyl stearate is hydrolysed to some extent to benzyl alcohol and stearic acid. In addition, several other *Mycena* species contain enzymes exhibiting an esterase activity. When we incubated the unnatural substrate benzyl stearate with the homogenate of *M. rosea* and *M. sanguinolenta*, at least one quarter of this ester was hydrolysed after 16 h. In contrast, the yeast *S. cerevisiae* was inactive and did not hydrolyse benzyl stearate at all.

Plants also contain lipases, which are activated upon wounding.[6] In plants free fatty acids are usually generated from lipids. The free fatty acids are subsequently degraded by lipoxygenases into compounds such as methyl jasmonate[6] and stimulate the production of volatiles that serve as signalling compounds in their chemical defence.[7] However, plants usually do not contain fatty acid esters of secondary metabolites as precursors for their chemical defence. In contrast, glycosides are often present in plants as inactive precursors. For instance, a number of plants hydrolyse cyanogenic glycosides upon injury and release hydrocyanic acid.[8]

A large number of secondary metabolites is already known from plants and higher fungi. However, the ecological roles of many compounds from fungi are still obscure. Well-known toxins from toadstools are the amanitins,[9] ibotenic acid[10] or orellanine,[11] which protect the fruiting bodies from feeding animals ranging from insects to mammals. Antifungal compounds such as the strobilurins, which have served as lead structures for the development of commercially available fungicides,[12] have been found in the mycelia of a variety of fungi including several *Mycena* species. Hydroxystrobilurin D (= hydroxystrobilurin G) from *M. sanguinolenta* is a benzodioxepine-type strobilurin[13] structurally related to the benzoxepines from *M. galopus*.

In contrast to the examples mentioned above, which represent secondary metabolites that are present permanently in their bioactive forms in fruiting bodies as a constitutive chemical defence, *M. galopus* seems to use a wound-activated defence mechanism based on the enzymatic conversion of an inactive precursor into the active agent that occurs only temporarily upon activation by injury. In comparison with direct chemical defence mechanisms relying on permanently present bioactive compounds, wound-activated chemical defence mechanisms have the advantage that the intact organism is not affected negatively by its own defence compounds.

Wound-activated defence mechanisms have been observed in mushrooms only in a limited number of cases: for instance, the fruiting bodies of *Aleurodiscus amorphus* contain the cyanohydrin ether aleurodisconitrile, which, upon injury of the fruiting bodies, is converted into highly toxic hydrocyanic acid and aleurodiscoester by an oxidative mechanism that differs fundamentally from the hydrolysis of cyanogenic glycosides occurring in a variety of plants.[14] Stephanosporin is a precursor of the fungicide 2-chloro-4-nitrophenol, which is generated in large quantities in the rare gasteromycete *Stephanospora caroticolor* after injury of the fruiting bodies.[15] In *Paxillus atrotomentosus* the leucomentins are converted into atromentin and the feeding deterrent osmundalactone after wounding of the fruiting bodies.[16]

Similarly to *M. galopus*, *Lactarius* species also contain a latex, which has been investigated in detail in a number of species. For instance, upon injury of *Lactarius vellereus*, fatty acid esters of the sesquiterpene velutinal that are present in the latex are saponified.[17] The unstable free velutinal is then transformed into pungent-tasting aldehydes such as velleral and isovelleral or the corresponding alcohols vellerol and isovellerol. In *Lactarius deliciosus* and *Lactarius deterrimus*, intact fruiting bodies exclusively contain a dihydroazulene alcohol that is esterified with stearic acid or linoleic acid. Upon injury the ester is hydrolysed, yielding a number of different pungent-tasting sesquiterpenes, such as lactaroviolin and delicial.[18]

Conclusion

The detection of a wound-activated chemical defence mechanism in *M. galopus* shows that chemical defence based on the cleavage of inactive fatty acid esters to active compounds is not restricted to some species of the genus *Lactarius*, but also occurs in a *Mycena* species. Moreover, the unspecific esterase activity upon injury of all tested *Mycena* species indicates that wound-activated chemical defence mechanisms might be more widespread than assumed previously. Secondary metabolites have often been isolated from mushrooms under conditions such as drying or homogenisation in aqueous solution, which might alter the metabolite pattern in relation to intact fruiting bodies. Thus, the presence of wound-activated defence mechanisms and of the corresponding inactive precursors might have been overlooked in some other cases. Hence, consequent metabolic profiling both of intact and artificially wounded species by GC-MS or LC-MS may lead to the detection of more wound-activated defence mechanisms in the future.

Experimental Section

General Experimental Procedures: Evaporation of the solvents was performed under reduced pressure with a rotary evaporator. Preparative HPLC separations were performed with two Waters 510 pumps equipped with an automated gradient controller 680 and an Applied Biosystems 783A UV/Vis detector. The samples were separated first on a Nucleodur C-18 EC, 5 μm, 10 × 250 mm column (Macherey–Nagel) with use of the following gradient program: from 49.97% H_2O/49.97% MeOH/0.06% AcOH over 20 min linear to 100% MeOH, then 15 min at 100% MeOH; flow rate: 6 mL min^{-1}; detection: UV at 230 nm. A second normal-phase separation ($HPLC_{NP}$) was performed with the same HPLC instrumentation and with a Nucleosil Si, 5 μm, 10 × 250 mm column (Macherey–Nagel) and use of the following isocratic separation conditions: 98% *n*-hexane, 2% *tert*-butyl methyl ether; flow rate: 6 mL min^{-1}; detection: UV at 280 nm. UV spectra were recorded with a Varian Cary 100 Bio UV/Vis spectrometer. NMR spectra were recorded with a Bruker DMX 250 spectrometer (^{1}H at 250.06, ^{13}C at 62.9 MHz), with a Bruker DMX 500 spectrometer equipped with a TXI probe (^{1}H at 500.11, ^{13}C at 125.8 MHz) and a Bruker

DMX 600 spectrometer equipped with a TXI cryo probe (^{1}H at 600.13, ^{13}C at 150.9 MHz). Chemical shifts were determined relative to the solvent $CDCl_3$ (δ_H = 7.26, δ_C = 77.0 ppm) as internal standard. GC-MS (EI) spectra were recorded with a Thermo Electron Trace DSQ mass spectrometer coupled with a Thermo Electron Trace GC Ultra equipped with a PTV injector. For sample separation, a fused silica DB 5 ms capillary column (15 m × 0.25 mm, coated with a 0.25 μm layer of liquid phase) and helium as carrier gas was used. Injection volumes were 0.2–0.5 μL of a 1–2% (m/v) solution. For pertrimethylsilylation, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was used. Temperature program: 1 min isothermal at 50 °C, then 5 °C min^{-1} up to 300 °C, finally 10 min isothermal at 300 °C. Retention indices R_i according to Kováts were determined by injection of a 0.2 μL sample of a standard mixture of saturated straight-chain alkanes (C_{10}–C_{36}). HRMS (APCI) spectra were obtained with a Thermo Scientific LTQ Orbitrap mass spectrometer. The spectrometer was operated in the positive mode (1 spectrum s^{-1}; mass range: 50–1000) with nominal mass resolving power of 60000 at *m*/*z* = 400 with a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation with polydimethylcyclosiloxane {[$(CH_3)_2SiO]_6$, *m*/*z* = 445.120025} as internal lock mass.

Mushrooms: Fruiting bodies of *M. galopus* (leg. et det. S. Peters and P. Spiteller) were collected in September and October 2004, 2005 and 2006 in coniferous forests near Leutstetten, 20 km south of Munich. Voucher samples of *M. galopus* are deposited at the Institut für Organische Chemie und Biochemie II der Technischen Universität München, Germany. The mushrooms were frozen with liquid nitrogen and stored at –35 °C after collecting.

Test Organisms: *Bacillus subtilis* (NCCB 1089), *Bacillus brevis* (NCCB 89173), *Escherichia coli* XL1 blue, *Cladosporium cucumerinum* (CBS 177.54, Centraalbureau voor Schimmelcultures, Amsterdam), *Spinellus fusiger* (CBS 633.80, Centraalbureau voor Schimmelcultures, Amsterdam, and FSU857, Max-Planck-Institut für Chemische Ökologie, Jena), *Saccharomyces cerevisiae* (Strain 6211, Lehrstuhl für Biotechnologie, Technische University of München, Garching).

Metabolic Profiling: For metabolic profiling of intact fruiting bodies of *M. galopus*, three freshly collected fruiting bodies were placed into MeOH (5 mL) immediately after collection in the forest. In order to monitor the hydrolytic cleavage of the benzoxepine esters, 18 freshly collected fruiting bodies of *M. galopus* were bruised with a spatula. After 0, 10, 30, 60, 180 and 1440 min, three of these fruiting bodies were placed into separate vials containing MeOH (5 mL). In order to monitor the stability of the benzoxepine esters in the latex, the latex of 15 freshly collected fruiting bodies of *M. galopus* was dripped immediately into six separated vials. After 0, 2, 10, 60 and 600 min, respectively, MeOH (5 mL) was applied to the vials. In the laboratory, the methanolic extract (1 mL) was removed from each sample, and the solvent was evaporated. Each sample was dissolved in AcOEt (200 μL) and dried with Na_2SO_4, and the solution was transferred to a new vial. The solvent was then removed in a stream of nitrogen, and each sample was trimethylsilylated with MSTFA (50 μL) and subjected to GC-MS analysis.

Extraction and Isolation of *E*-1, *Z*-1 and 2: Frozen fruiting bodies (40 g) were crushed after addition of AcOEt (25 mL) and extracted twice with AcOEt (2 × 25 mL) at 25 °C for 30 min. The combined extracts were then concentrated in vacuo at 40 °C. The resulting residue was heated in methanolic KOH (0.02 M, 5 mL) at 70 °C for 5 h. After concentration in vacuo at 40 °C, the residue was dissolved in water (5 mL) and extracted with AcOEt (3 × 5 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated in vacuo at 40 °C. The resulting residue was dissolved in MeOH (5 mL), prepurified with an RP-18 cartridge and separated on an RP-18 column by semipreparative HPLC, yielding three benzoxepine-containing fractions. The first fraction at R_t = 8.9 min (1.3 mg) consisted of **2**, the second at R_t = 11.1 min (1.9 mg) consisted of pure ***E*-1**, while the third fraction at R_t = 11.4 min (2.2 mg) consisted of a 1:1 mixture of ***Z*/*E*-1**.

Extraction and Isolation of *E*/*Z*-1a to *E*/*Z*-1e and 2a–2e: Frozen fruiting bodies (15 g) were crushed after addition of AcOEt (25 mL) and extracted twice with AcOEt (2 × 25 mL) at 25 °C for 30 min. The combined extracts were then concentrated in vacuo at 40 °C. The resulting residue was dissolved in MeOH (5 mL), prepurified with an RP-18 cartridge and separated on an RP-18 column by preparative HPLC (UV detection at 230 nm) yielding two benzoxepine-containing fractions. The first fraction at R_t = 26.5 min (1.1 mg) consisted of ***E*/*Z*-1b**, ***E*/*Z*-1e**, **2b** and **2e**, while the second one eluting at R_t = 28.2 min (1.7 mg) consisted of ***E*/*Z*-1a**, ***E*-1d** and **2a**. After that, both fractions were separated by preparative HPLC (UV detection at 280 nm) on a normal-phase silica gel column. From a sample (100 g) of frozen fruiting bodies of *M. galopus*, ***E*-1b** (1.0 mg), ***Z*-1b** (0.5 mg), ***E*-1a** (5.7 mg), ***Z*-1a** (1.8 mg), ***E*-1e** (2.0 mg), ***Z*-1e** (0.7 mg), ***E*-1d** (2.5 mg), **2a** (1.7 mg) and **2e** (0.7 mg) were isolated.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methanol (*E*-1): Colourless solid. $HPLC_{RP18}$: R_t = 11.1 min. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ = 4.55 (s, 2 H, 2-H), 4.65 (s, 2 H, 11-H), 6.12 (s, 1 H, 10-H), 6.55 (d, *J* = 11.8 Hz, 1 H, 4-H), 6.83 (d, *J* = 11.8 Hz, 1 H, 5-H), 6.99 (d, *J* = 8.2 Hz, 1 H, 9-H), 7.19 (dd, *J* = 8.2, 2.0 Hz, 1 H, 8-H), 7.27 (d, *J* = 2.0 Hz, 1 H, 6-H) ppm.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Trimethylsilyl Ether (Trimethylsilyl Derivative of *E*-1): GC-MS: R_i = 2117; *m*/*z* (%) = 296 (22) $[M(^{37}Cl)]^+$, 294 (64) $[M(^{35}Cl)]^+$, 281 (3), 279 (9), 259 (13), 251 (3), 249 (11), 243 (5), 231 (3), 229 (5), 207 (34) $[M(^{37}Cl) - OSi(CH_3)_3]^+$, 205 (100) $[M(^{35}Cl) - OSi(CH_3)_3]^+$, 185 (8), 170 (44), 169 (39), 157 (4), 142 (16), 141 (63), 139 (11), 131 (16), 128 (11), 122 (12), 115 (30), 77 (9), 75 (13), 73 (48), 59 (5), 45 (11).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Trimethylsilyl Ether (Trimethylsilyl Derivative of *Z*-1): GC-MS: R_i = 2105; *m*/*z*: see trimethylsilylated ***E*-1**.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Palmitate (*E*-1a): Colourless solid. $HPLC_{RP18}$: R_t = 28.2 min. $HPLC_{NP}$: R_t = 10.2 min. ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 1. UV/Vis (H_2O): λ_{max} (lg ε) = 239 (4.43), 289 (4.44) nm. GC-MS: R_i = 3622; *m*/*z* (%) = 462 (6) $[M(^{37}Cl)]^+$, 460 (16) $[M(^{35}Cl)]^+$, 224 (8) $[M(^{37}Cl) - O{=}C{=}CH(CH_2)_{13}CH_3]^+$, 222 (23) $[M(^{35}Cl) - O{=}C{=}CH(CH_2)_{13}CH_3]^+$, 207 (35) $[M(^{37}Cl) - O_2C(CH_2)_{14}CH_3]^+$, 205 (100) $[M(^{35}Cl) - O_2C(CH_2)_{14}CH_3]^+$, 171 (20), 170 (29), 141 (18), 131 (4), 128 (4), 115 (7), 69 (6), 57 (5), 55 (8), 43 (9), 41 (8).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Palmitate (*Z*-1a): Colourless solid. $HPLC_{RP18}$: R_t = 28.2 min. $HPLC_{NP}$: R_t = 9.5 min. GC-MS: R_i = 3611; *m*/*z*: see ***E*-1a**.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Palmitoleate (*E*-1b): Colourless solid. $HPLC_{RP18}$: R_t = 26.5 min. $HPLC_{NP}$: R_t = 10.6 min. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 0.88 (t, *J* = 6.3 Hz, 3 H, 16′-H), 1.26–1.31 (m, 16 H, 4′-H to 7′-H and 12′-H to 15′-H), 1.63 (tt, *J* = 7.6, 7.6 Hz, 2 H, 3′-H), 2.00–2.01 (m, 4 H, 8′-H, 11′-H), 2.34 (t, *J* = 7.6 Hz, 2 H, 2′-H), 4.55 (s, 2 H, 2-H), 5.05 (s, 2 H, 11-H), 5.33–5.35 (m, 2 H, 9′-H, 10′-H),

6.12 (s, 1 H, 10-H), 6.53 (d, J = 11.8 Hz, 1 H, 5-H), 6.83 (d, J = 11.8 Hz, 1 H, 4-H), 6.98 (d, J = 8.8 Hz, 1 H, 9-H), 7.18 (d, J = 8.8 Hz, 1 H, 8-H), 7.26 (s, 1 H, 6-H) ppm. HRMS (APCI): calcd. for $C_{28}H_{40}{}^{35}ClO_3$ 459.2666, found 459.2660 $[M + H]^+$. GC-MS: R_i = 3609; *m*/*z* (%) = 460 (1) $[M(^{37}Cl)]^+$, 458 (3) $[M(^{35}Cl)]^+$, 224 (2) $[M(^{37}Cl) - O{=}C{=}CH(C_{14}H_{27})]^+$, 222 (6) $[M(^{35}Cl) - O{=}C{=}CH(C_{14}H_{27})]^+$, 207 (38) $[M(^{37}Cl) - O_2C(CH_2)_7CHCH(CH_2)_5CH_3]^+$, 205 (100) $[M(^{35}Cl) - O_2C(CH_2)_7CHCH(CH_2)_5CH_3]^+$, 171 (14), 170 (22), 141 (12), 131 (4), 128 (4), 115 (6), 69 (7), 55 (9), 43 (6), 41 (7).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Palmitoleate (*Z*-1b): Colourless solid. $HPLC_{RP18}$: R_t = 26.5 min. $HPLC_{NP}$: R_t = 9.8 min. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ = 0.88 (t, J = 7.1 Hz, 3 H, 16′-H), 1.24–1.33 (m, 16 H, 4′-H to 7′-H and 12′-H to 15′-H), 1.63 (tt, J = 7.2, 7.2 Hz, 2 H, 3′-H), 2.33 (t, J = 7.2 Hz, 2 H, 2′-H), 2.00–2.01 (m, 4 H, 8′-H, 11′-H), 4.86 (s, 2 H, 2-H), 5.04 (s, 2 H, 11-H), 5.33–5.34 (m, 2 H, 9′-H, 10′-H), 6.33–6.34 (m, 3 H, 4-H, 5-H, 10-H), 7.01 (d, J = 8.6 Hz, 1 H, 9-H), 7.16 (dd, J = 8.6, 2.2 Hz, 1 H, 8-H), 7.18 (d, J = 2.2 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 298 K): δ = 14.1 (C-16′), 22.7 (C-15′), 25.0 (C-3′), 27.2 (C-8′ and C-11′) 29.1–29.8 (C-4′–C-7′ and C-12′–C-13′), 31.9 (C-14′) 34.3 (C-2′), 65.4 (C-11), 68.1 (C-2), 120.2 (C-10), 120.3 (C-9), 127.7 (C-7), 127.8 (C-4 or C-5), 128.1 (C-5 or C-4), 128.9 (C-8), 129.7 (C-9′ or C-10′), 130.0 (C-10′ or C-9′), 131.0 (C-5a), 132.4 (C-6), 138.2 (C-3), 159.0 (C-9a) 173.7 (C-1′) ppm. GC-MS: R_i = 3593; *m*/*z*: see ***E*-1b**.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Stearate (*E*-1c): GC-MS: R_i = 3861; *m*/*z* (%) = 490 (14) $[M(^{37}Cl)]^+$, 488 (32) $[M(^{35}Cl)]^+$, 279 (2), 277 (2), 224 (16) $[M(^{37}Cl) - O{=}C{=}CH(CH_2)_{15}CH_3]^+$, 222 (47) $[M(^{35}Cl) - O{=}C{=}CH(CH_2)_{15}CH_3]^+$, 207 (38) $[M(^{37}Cl) - O_2C(CH_2)_{15}CH_3]^+$, 205 (100) $[M(^{35}Cl) - O_2C(CH_2)_{15}CH_3]^+$, 171 (33), 170 (46), 141 (25), 131 (10), 128 (4), 115 (10), 69 (11), 67 (10), 57 (14), 55 (14).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Stearate (*Z*-1c): GC-MS: R_i = 3847; *m*/*z*: see ***E*-1c**.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Oleate (*E*-1d): Colourless solid. $HPLC_{RP18}$: R_t = 28.2 min. $HPLC_{NP}$: R_t = 10.6 min. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 0.88 (t, J = 6.7 Hz, 3 H, 18′-H), 1.27–1.29 (m, 20 H, 4′-H to 7′-H and 12′-H to 17′-H), 1.64 (tt, J = 7.6, 7.6 Hz, 2 H, 3′-H), 1.98–2.03 (m, 4 H, 8′-H, 11′-H), 2.34 (t, J = 7.6 Hz, 2 H, 2′-H), 4.55 (s, 2 H, 2-H), 5.05 (s, 2 H, 11-H), 5.32–5.37 (m, 2 H, 9′-H, 10′-H), 6.12 (s, 1 H, 10-H), 6.53 (d, J = 11.9 Hz, 1 H, 5-H), 6.83 (d, J = 11.9 Hz, 1 H, 4-H), 6.98 (d, J = 8.0 Hz, 1 H, 9-H), 7.18 (dd, J = 8.0, J = 2.4 Hz, 1 H, 8-H), 7.26 (d, J = 2.4 Hz, 1 H, 6-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, 298 K): δ = 14.1 (C-18′), 22.7 (C-17′), 25.0 (C-3′), 27.2 (C-8′ and C-11′) 29.1–29.8 (C-4′–C-7′ and C-12′–C-15′), 31.9 (C-16′) 34.3 (C-2′), 65.4 (C-11), 72.6 (C-2), 119.4 (C-10), 120.3 (C-9), 124.2 (C-4), 127.0 (C-7), 129.4 (C-8), 129.7 (C-9′ or C-10′), 130.0 (C-10′ or C-9′), 130.8 (C-5a), 130.9 (C-5), 133.4 (C-6), 136.4 (C-3), 159.2 (C-9a) 173.6 (C-1′) ppm. HRMS (APCI): calcd. for $C_{30}H_{44}{}^{35}ClO_3$ 487.2979, found 487.2977 $[M + H]^+$. GC-MS: R_i = 3835; *m*/*z* (%) = 488 (0.3) $[M(^{37}Cl)]^+$, 486 (1) $[M(^{35}Cl)]^+$, 279 (6), 277 (4), 224 (1) $[M(^{37}Cl) - O{=}C{=}CH(C_{16}H_{31})]^+$, 222 (4) $[M(^{35}Cl) - O{=}C{=}CH(C_{16}H_{31})]^+$, 207 (38) $[M(^{37}Cl) - O_2C(C_{17}H_{33})]^+$, 205 (100) $[M(^{35}Cl) - O_2C(C_{17}H_{33})]^+$, 171 (12), 170 (20), 141 (10), 131 (3), 128 (3), 115 (4), 95 (3), 81 (4), 69 (5), 67 (10), 55 (7), 43 (4), 41 (6).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Oleate (*Z*-1d): GC-MS: R_i = 3819; *m*/*z*: see ***E*-1d**.

{(*E*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Linoleate (*E*-1e): Colourless solid. $HPLC_{RP18}$: R_t = 26.5 min. $HPLC_{NP}$: R_t = 11.0 min. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ = 0.89 (t, J = 6.6 Hz, 3 H, 18′-H), 1.29–1.30 (m, 10 H, 4′-H to 6′-H, 16′-H, 17′-H), 1.33–1.37 (m, 4 H, 7′-H, 15′-H), 1.63 (tt, J = 7.4, 7.4 Hz, 2 H, 3′-H), 2.03–2.04 (m, 4 H, 8′-H, 14′-H), 2.34 (t, J = 7.4 Hz, 2 H, 2′-H), 2.77 (dd, J = 7.1, 7.1 Hz, 2 H, 11′-H), 4.55 (s, 2 H, 2-H), 5.05 (s, 2 H, 11-H), 5.30–5.35 (m, 2 H, 10′-H, 12′-H), 5.35–5.40 (m, 2 H, 9′-H, 13′-H), 6.12 (s, 1 H, 10-H), 6.53 (d, J = 11.8 Hz, 1 H, 5-H), 6.83 (d, J = 11.8 Hz, 1 H, 4-H), 6.98 (d, J = 8.4 Hz, 1 H, 9-H), 7.18 (d, J = 8.4 Hz, 1 H, 8-H), 7.26 (s, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 298 K): δ = 14.1 (C-18′), 22.6 (C-17′), 24.9 (C-3′), 25.6 (C-11′), 27.2 (C-8′ and C-14′), 29.1–29.7, (C-4′ to C-7′ and C-15′), 31.5 (C-16′), 34.3 (C-2′), 65.4 (C-11), 72.6 (C-2), 119.4 (C-10), 120.3 (C-9), 124.2 (C-4), 127.0 (C-7), 127.9 (C-10′ or C-12′), 128.0 (C-12′ or C-10′), 129.5 (C-8), 130.0 (C-9′ or C-13′), 130.2 (C-13′ or C-9′), 130.8 (C-5a), 130.9 (C-5), 133.4 (C-6), 136.3 (C-3), 159.2 (C-9a), 173.7 (C-1′) ppm. GC-MS: R_i = 3834; *m*/*z* (%) = 486 (0.3) $[M(^{37}Cl)]^+$, 484 (1) $[M(^{35}Cl)]^+$, 279 (12), 277 (7), 224 (1) $[M(^{37}Cl) - O{=}C{=}CH(C_{16}H_{29})]^+$, 222 (4) $[M(^{35}Cl) - O{=}C{=}CH(C_{16}H_{29})]^+$, 207 (43) $[M(^{37}Cl) - O_2C(C_{17}H_{31})]^+$, 205 (100) $[M(^{35}Cl) - O_2C(C_{17}H_{31})]^+$, 171 (18), 170 (26), 141 (14), 131 (4), 128 (4), 115 (6), 95 (6), 81 (6), 67 (7), 55 (6).

{(*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Linoleate (*Z*-1e): Colourless solid. R_t = 26.5 min. $HPLC_{NP}$: R_t = 10.2 min. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ = 0.88 (t, J = 7.4 Hz, 3 H, 18′-H), 1.29–1.37 (m, 14 H, 4′-H to 7′-H and 15′-H to 17′-H), 1.63 (tt, J = 6.4, 6.4 Hz, 2 H, 3′-H), 2.00–2.05 (m, 4 H, 8′-H, 14′-H), 2.34 (t, J = 6.4 Hz, 2 H, 2′-H), 2.77 (dd, J = 6.9, 6.9 Hz, 2 H, 11′-H), 4.86 (s, 2 H, 2-H), 5.05 (s, 2 H, 11-H), 5.31–5.35 (m, 2 H, 10′-H, 12′-H), 5.34–5.38 (m, 2 H, 9′-H, 13′-H), 6.33–6.34 (m, 3 H, 4-H, 5-H, 10-H), 7.01 (d, J = 8.5 Hz, 1 H, 9-H), 7.17 (d, J = 8.5 Hz, 1 H, 8-H), 7.21 (s, 1 H, 6-H) ppm. GC-MS: R_i = 3815; *m*/*z*: see ***E*-1e**.

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methanol (2): Colourless solid. $HPLC_{RP18}$: R_t = 8.9 min. ^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ = 4.58 (s, 2 H, 2-H), 4.64 (s, 2 H, 11-H), 5.07 (s, 1 H, 10-H_b), 5.23 (s, 1 H, 10-H_a), 6.35 (d, J = 11.6 Hz, 1 H, 5-H), 6.47 (d, J = 11.6 Hz, 1 H, 4-H), 6.99 (d, J = 8.2 Hz, 1 H, 9-H), 7.16 (dd, J = 8.2, 2.0 Hz, 1 H, 8-H), 7.24 (d, J = 2.0 Hz, 1 H, 6-H) ppm.

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Trimethylsilyl Ether (Trimethylsilyl Derivative of 2): GC-MS: R_i = 1864; *m*/*z* (%) = 260 (64) $[M]^+$, 245 (10), 215 (13), 186 (4), 185 (5), 171 (100) $[M - OSi(CH_3)_3]^+$, 141 (13), 131 (6), 128 (19), 115 (10), 75 (6), 73 (6).

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Palmitate (2a): Colourless solid. $HPLC_{RP18}$: R_t = 28.2 min. $HPLC_{NP}$: R_t = 8.4 min. ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 1. UV/Vis (H_2O): λ_{max} (lg ε) = 236 (4.42), 282 (4.34) nm. GC-MS: R_i = 3353; *m*/*z* (%) = 426 (11) $[M]^+$, 188 (42) $[M - O{=}C{=}CH(CH_2)_{13}CH_3]^+$, 172 (78), 171 (100) $[M - O_2C(CH_2)_{14}CH_3]^+$, 159 (6), 157 (8), 141 (8), 131 (5), 128 (15), 115 (6), 57 (6), 55 (6), 43 (10), 41 (6).

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Palmitoleate (2b): Colourless solid. $HPLC_{RP18}$: R_t = 26.5 min. $HPLC_{NP}$: R_t = 8.6 min. GC-MS: R_i = 3336; *m*/*z* (%) = 424 (1) $[M]^+$, 188 (7) $[M - O{=}C{=}CHC_{14}H_{27}]^+$, 172 (83) 171 (100) $[M - O_2C(CH_2)_7CHCH(CH_2)_5CH_3]^+$, 157 (4), 141 (4), 131 (3), 128 (11), 115 (4), 69 (4), 55 (6), 43 (4), 41 (5).

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Stearate (2c): GC-MS: R_i = 3545; *m*/*z* (%) = 454 (6) $[M]^+$, 188 (26) $[M - O_2C(CH_2)_{16}CH_3]^+$, 172 (88), 171 (100) $[M - O_2C(CH_2)_{16}CH_3]^+$, 157 (8), 131 (6), 128 (14), 115 (6), 57 (86), 55 (6), 43 (12).

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Oleate (2d): GC-MS: R_i = 3543; *m*/*z* (%) = 452 (1) $[M]^+$, 281 (3), 277 (6), 188

(6) [M – O=C=CH–$(CH_2)_6$CHCH$(CH_2)_7CH_3$]$^+$, 172 (85), 171 (100) [M – $O_2CC_{17}H_{33}$]$^+$, 157 (6), 141 (9), 131 (6), 128 (17), 115 (6), 55 (9).

(2,3-Dihydro-3-methylenebenzo[*b*]oxepin-7-yl)methyl Linoleate (2e): Colourless solid. $HPLC_{RP18}$: R_t = 26.5 min. $HPLC_{NP}$: R_t = 9.0 min. GC-MS: R_i = 3538; *m*/*z* (%) = 450 (1) [M]$^+$, 277 (8), 188 (4) [M – O=C=C$HC_{16}H_{29}$]$^+$, 172 (64), 171 (100) [M – $O_2CC_{17}H_{31}$]$^+$, 157 (5), 141 (5), 128 (13), 115 (3), 67 (4), 55 (4).

1-{3-(Chloromethylene)-6-hydroxy-2,3-dihydrobenzo[*b*]oxepin-7-yl}ethanone (3): GC-MS: R_i = 2180; *m*/*z* (%) = 252 (31) [M(^{37}Cl)]$^+$, 250 (100) [M(^{35}Cl)]$^+$, 237 (4), 235 (15), 215 (38), 213 (15), 201 (6), 199 (6), 197 (8), 179 (6), 173 (16), 171 (7), 161 (29), 141 (6), 115 (18), 113 (4), 105 (13), 77 (6), 43 (12).

Hydrolysis of the Isolated Benzoxepine Esters: Pure samples (0.5 mg) of ***E*-1a**, ***Z*-1a** and **2a** were dissolved in a methanolic KOH solution (0.02 M, 2 mL) and heated to 60 °C for 1 h. The solvent was removed at 40 °C in vacuo, and the residue was acidified with HCl (0.1 N, 1 mL) and extracted with CH_2Cl_2 (2 × 1 mL). The organic phase was dried with Na_2SO_4, and the solvent was removed at 40 °C in vacuo. The residue was then either trimethylsilylated with MSTFA or methylated with CH_2N_2. The samples were subjected to GC-MS analysis. For the identification of the fatty acids, authentic samples of the corresponding derivatives were compared by GC-MS with the derivatives of the compounds originating from *M. galopus*.

Biological Tests: For plate diffusion assays, either ***E*/*Z*-1** (100 or 500 μg), or a mixture of ***E*/*Z*-1a**, ***E*/*Z*-1c**, **2a** and **2c** (100 or 500 μg, first fraction of the HPLC separation on RP-18 at R_t = 26.5 min), or a mixture of ***E*/*Z*-1b**, ***E*-1d** and **2b** (100 or 500 μg, second fraction of the HPLC separation on RP-18 at R_t = 28.2 min), was dissolved in AcOEt (50 μL) and dropped onto paper discs (Ø 6 mm, thickness 0.5 mm). These discs were dried under sterile conditions and placed on agar plates inoculated with the test organism (*Bacillus brevis*, *Bacillus subtilis*, *Escherichia coli*, *Cladosporium cucumerinum* and *Spinellus fusiger*). The plates were incubated in the case of bacteria at 37 °C for 24 h, in the case of *C. cucumerinum* at 18 °C for 48 h and in the case of *S. fusiger* at 18 °C for 96 h. The bioassay against the yeast *Saccharomyces cerevisiae* was performed in liquid culture according to Wijnberg.[3b] Either ***E*-1a** (0.23 mg or 0.50 μmol), ***E*-1e** (0.24 mg or 0.50 μmol), a mixture of ***E*/*Z*-1b**, ***E*/*Z*-1e**, **2b** and **2e** (0.23 mg, 0.50 μmol, first fraction of the HPLC separation on RP-18 at R_t = 26.5 min), a mixture of ***E*/*Z*-1a**, ***E*-1d** and **2a** (0.23 mg, 0.50 μmol, second fraction of the HPLC separation on RP-18 at R_t = 28.2 min), ***E*/*Z*-1** (0.11 mg or 0.50 μmol, 1:1 mixture), ***E*-1** (0.11 mg or 0.50 μmol) or nourseothricin (NTC, 0.5 μmol) was dissolved in CH_2Cl_2 (50 μL) and placed in a sterile 5 mL test tube. After evaporation of the CH_2Cl_2, liquid YMG medium (500 μL) consisting of yeast extract (0.4%, w/v), malt extract (1.0%, w/v) and glucose (0.4%, w/v) was added, and each test tube was inoculated with cells (5 μL) from a freshly prepared 24 h preculture of *S. cerevisiae* and incubated in a shaking cabinet at 30 °C. After 18 h, the yeast cell concentration was determined by reading the optical density at 660 nm. A pure yeast culture, grown in the absence of test compounds, served as reference for the determination of the relative growth. The test was repeated three times for each compound and for the control. Relative growth rates: see Figure 3.

{(*E*/*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Acetate: Compound ***E*/*Z*-1** (0.5 mg), pyridine (1 mL) and acetic anhydride (100 μL) were heated at 100 °C for 2 h. The solvent and the excess of acetic anhydride were then removed at 40 °C in vacuo. Yield (GC-MS): 99%. Colourless solid. GC-MS: R_i[(*Z*) isomer] = 2132, R_i[(*E*) isomer] = 2146; *m*/*z* (%) = 266 (25) [M(^{37}Cl)]$^+$, 264 (65) [M(^{35}Cl)]$^+$, 229 (38) [M – Cl]$^+$, 224 (15), 222 (52), 207 (9) [M(^{37}Cl) – CH_3CO_2]$^+$, 205 (22) [M(^{35}Cl) – CH_3CO_2]$^+$, 187 (27), 170 (52), 169 (61), 157 (13), 141 (100), 139 (12), 131 (31), 128 (41), 115 (48), 43 (37).

{(*E*/*Z*)-3-(Chloromethylene)-2,3-dihydrobenzo[*b*]oxepin-7-yl}methyl Caprylate: Compound ***E*/*Z*-1** (0.5 mg), octanoic acid (10 μL) and $(CH_3)_3$SiCl (2 μL) were heated at 80 °C for 3 h. Then, $(CH_3)_3$SiCl and the excess of octanoic acid were removed at 80 °C at 0.1 mbar. Yield (GC-MS): 99%. Colourless solid. GC-MS: R_i[(*Z*) isomer] = 2741, R_i[(*E*) isomer] = 2754; *m*/*z* (%) = 350 (18) [M(^{37}Cl)]$^+$, 348 (48) [M(^{35}Cl)]$^+$, 313 (7) [M – Cl]$^+$, 224 (31), 222 (93), 207 (39) [M(^{37}Cl) – $C_7H_{15}CO_2$]$^+$, 205 (100) [M(^{35}Cl) – $C_7H_{15}CO_2$]$^+$, 187 (13), 170 (81), 169 (56), 157 (14), 141 (89), 131 (17), 128 (25), 115 (39), 57 (35), 55 (16), 43 (18), 41 (20).

Benzyl Stearate: Stearic acid (100 mg), benzyl alcohol (0.5 mL) and $(CH_3)_3$SiCl (10 μL) were heated at 130 °C for 1 h. After removal of the benzyl alcohol in vacuo, the reaction product was purified on a silica gel column (10 × 1 cm) with hexane/AcOEt (4:1) as eluent. Yield: 112.5 mg (85%). Colourless solid. ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ = 0.91 (t, *J* = 6.5 Hz, 3 H, CH_3), 1.15–1.42 (m, 28 H, 14 × CH_2), 1.67 (tt, *J* = 7.5, 7.0 Hz, 2 H, CH_2), 2.37 (t, *J* = 7.5 Hz, 2 H, CH_2), 5.14 (s, 2 H, CH_2), 7.27–7.43 (m, 5 H, 5 × CH) ppm. ^{13}C NMR (63 MHz, $CDCl_3$, 298 K): δ = 14.1 (CH_3), 22.7 (CH_2), 24.9 (CH_2), 29.1–29.8 (12 × CH_2), 31.9 (CH_2), 34.3 (CH_2), 66.0 (CH_2), 128.06 (C_q), 128.08 (CH), 128.4 (CH), 136.1 (C_q), 173.5 (C_q) ppm. GC-MS: R_i = 2791; *m*/*z* (%) = 374 (1) [M]$^+$, 283 (4) [M – C_7H_7]$^+$, 265 (5), 247 (3), 139 (3), 125 (4), 111 (6), 108 (69), 91 (100) [C_7H_7]$^+$, 85 (8), 83 (8), 81 (4), 79 (5), 77 (3), 71 (11), 69 (8), 65 (5), 57 (18), 55 (12), 43 (19).

Esterase Activity of *M. galopus*: Frozen fruiting bodies (1 g) were crushed, H_2O (1 mL) and either acetylated ***E*/*Z*-1**, caprylated ***E*/*Z*-1**, methyl oleate or benzyl stearate [100 μg dissolved in EtOH (20 μL)] were added, and the mixture was incubated at 25 °C and shaken vigorously. After 24 h, the mixture was extracted with $CHCl_3$ (2 × 1 mL). The organic phase was dried with Na_2SO_4, the solvent was removed at 40 °C in vacuo, and the residue was trimethylsilylated with MSTFA and subjected to GC-MS analysis. Yields (GC-MS): 99% (starting compound: acetate of ***E*/*Z*-1**, caprylate of ***E*/*Z*-1**, methyl oleate), 6% (starting compound: benzyl stearate). In control experiments, in which $CHCl_3$ (1 mL) was added to the mixture instead of H_2O, no esterase activity was detectable by GC-MS.

Esterase Activity of Selected *Mycena* Species: Frozen fruiting bodies (1 g) were crushed, H_2O (1 mL) and benzyl stearate [100 μg dissolved in EtOH (20 μL)] were added, and the mixture was incubated at 25 °C. After 16 h, the mixture was extracted with AcOEt (2 × 1 mL). The organic phase was dried with Na_2SO_4, the solvent was removed at 40 °C in vacuo, and the residue was trimethylsilylated with MSTFA and subjected to GC-MS analysis. Yields of free benzyl alcohol (GC-MS): 32% (*M. sanguinolenta*), 24% (*M. rosea*), 6% (*M. galopus*), 3% (*Mycena aurantiomarginata*).

Supporting Information (see also the footnote on the first page of this article): Selected NMR, UV/Vis and mass spectra of ***E*/*Z*-1**, ***E*/*Z*-1a** to ***E*/*Z*-1e**, **2**, **2a**–**2e** and **3**.

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