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New Benzodioxepin Type Strobilurins from Basidiomycetes. Structural Revision and Determination of the Absolute Configuration of Strobilurin D and Related β-Methoxyacrylate Antibiotics

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Abstract

The new antifungal strobilurins I (**1**) and K (**19**) are 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin derivatives. Their structure and stereochemistry was determined by degradation to aldehyde **3**. Both enantiomers of **3** were synthesised and the absolute configurations assigned by the high-field ^{1}H NMR variant of Mosher's method. (*S*)-**3** is identical with the compound derived from the natural products. In the course of these investigations the epoxide structures **15**, **16** and **17** previously assigned to strobilurin D, hydroxystrobilurin D and 9-methoxystrobilurin K have to be changed in **18**, **21** and **20**, respectively. All these compounds possess the same benzodioxepin core structure and (*S*)-configuration as strobilurin I (**1**).

Keywords: Strobilurins, Benzodioxepins, Antifungals, Natural Products, Regiochemistry

The strobilurins are an important class of antifungal antibiotics[1] which have served as lead compounds for the development of a new generation of industrial fungicides.[2] Recently, we isolated strobilurin D[3] and a new fungicide named strobilurin I (**1**) from cultures of an unidentified *Agaricus* species from Ethiopia.

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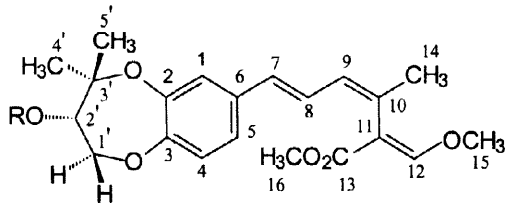
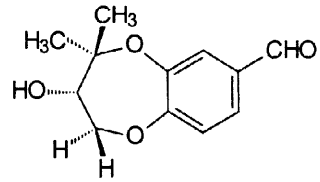
Strobilurin I (1)

The high resolution mass spectrum of strobilurin I indicated the molecular formula $C_{21}H_{26}O_6$. The strobilurin side chain was recognised by the usual NMR signals (Table 1) and characteristic fragment ions at *m/z* 237 ($C_{13}H_{17}O_4$) and 153 ($C_8H_9O_3$) in the EI mass spectrum.[4] The NMR spectra indicated the presence of a 1,2,4-trisubstituted benzene ring to which a $C_5H_{10}O$-unit was attached by two oxygen atoms (Table 1).



1, R = H
2, R = OAc



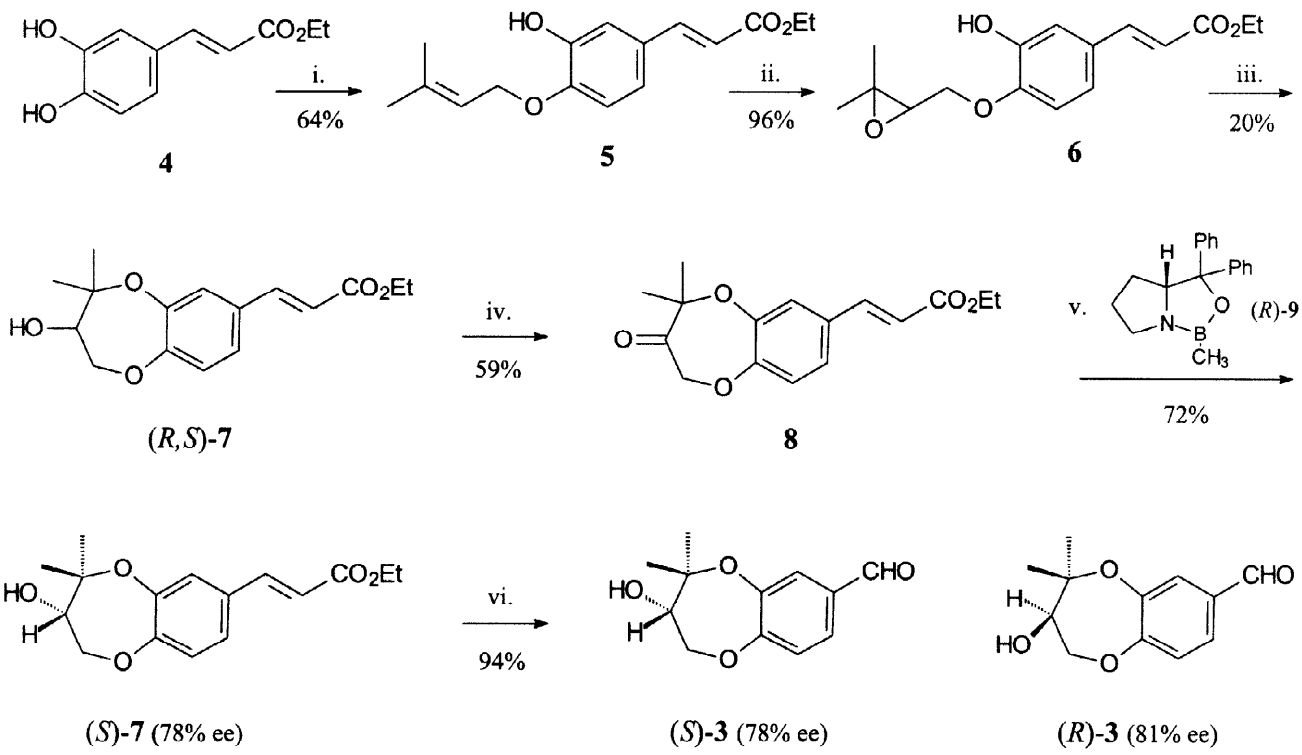
(*S*)-**3**

Table 1. ^1H and ^{13}C NMR data of strobilurin I (**1**) and aldehyde **3** in [D_6]acetone

position	**1**				**3**			
	δ_C [ppm]	$^nJ_{CH}$ [Hz]	δ_H [ppm]		δ_C [ppm]	$^nJ_{CH}$ [Hz]	δ_H [ppm]	
1	122.39	Dm (158)	6.96	d	124.70	Dd (162.0, 7.5)	7.45	d
2	148.66	m			147.35	ddd (7.1, 4.0, 1.5)		
3	152.15	m			156.78	m		
4	121.85	Dm (158)	6.84	d	121.08	Dd (160.0, 0.9)	7.08	d
5	122.75	Dm (158)	6.99	dd	125.79	Ddd (162.6, 7.6, 2.0)	7.55	dd
6	134.87	m			132.54	dd (24.6, 8.6)		
7	130.47	Dm (150)	6.38	d	190.16	Ddd (175.0, 4.7)	9.88	s
8	127.19	Dm (148)	6.56	dd				
9	130.37	Dm (151)	6.14	dq				
10	131.98	m						
11	111.06	m						
12	160.00	Dm (181)	7.45	s				
13	167.68	m						
14	23.88	Qd (127, 6.5)	1.89	s				
15	62.07	Qd (146, 6.5)	3.87	s				
16	51.35	Q (147)	3.64	s				
1'	72.26	T (145)	a: 3.89	dd	71.63	Td (146.6, 2.4)	a: 4.10	dd
			b: 4.14	dd			b: 4.32	dd
2'	75.65	Dm (141)	3.75	ddd	74.21	Dm (141.0)	3.87	ddd
3'	81.27	m			81.47	m		
4'	21.94	Qm (127)	1.24	s	20.98	Qm (126.8)	1.30*	s
5'	26.88	Qm (126)	1.36	s	25.95	Qm (126.8)	1.41*	s
OH			4.27	d			4.50	d

1: *J*[Hz]: 1–5 = 2.2; 4–5 = 8.2; 7–8 = 15.6; 8–9 = 10.7; 9–14 = 0.7; 1'a–1'b = 12.3; 1'a–2' = 7.2; 1'b–2' = 3.0; 2'–OH = 7.3.
3: *J*[Hz]: 1–5 = 2.1; 4–5 = 8.2; 1'a–1'b = 12.3; 1a–2' = 6.9; 1'b–2' = 3.0; 2'–OH = 7.0.
* The methyl groups at C-3' were assigned according to the strong nuclear Overhauser effects observed between 4'-CH_3 and OH, and 5'-CH_3 and H-2', respectively.

The downfield shift of the methine signal 2'-H from δ 3.75 to 5.00 after conversion of **1** into its acetate **2** is in accord with a 1,4-benzodioxepin structure[5] and excludes structural proposals with 1,4-dioxane or oxirane rings. In order to determine the structure and stereochemistry of strobilurin I (**1**), the compound was degraded by ozonolysis. The resulting optically active aldehyde **3** was then compared with the enantiomers of **3** obtained by stereoselective syntheses (Scheme 1).



Scheme 1. Synthesis of aldehyde (*S*)-**3**. Reagents and conditions: (i). 3,3-dimethylallyl bromide, K_2CO_3, DMF, 0 °C. (ii). *m*CPBA, CH_2Cl_2. (iii). La(OTf)$_3$ (0.25 equ), H_2O (50 equ), CH_2Cl_2, 25 °C, 7 d. (iv). PCC, CH_2Cl_2 (v). (*R*)-**9** (0.03 equ), $BH_3 \times Me_2S$ (0.6 equ), THF, 0 °C, 20 min (vi). 1. O_3, CH_2Cl_2, -78 °C; 2. Me_2S, 25 °C. Yields relate to chromatographically pure compounds.

The enantioselective synthesis of **3** starts from ethyl (*E*)-3,4-dihydroxycinnamate (**4**), which was alkylated with 3,3-dimethylallyl bromide to afford the 4-prenyl ether **5**. Oxidation of **5** with *m*-chloroperbenzoic acid (*m*CPBA) yielded the epoxy derivative **6**, whose cyclisation to the desired benzodioxepin **7** proved to be difficult. After several trials,[6,7] we obtained **7** in 20% yield by treatment of epoxide **6** with a catalytic amount of lanthanium(III)triflate[8] in dichloromethane and water. Oxidation of (*R,S*)-**7** with pyridinium chlorochromate (PCC)[9] yielded the benzodioxepinone **8**, which on reduction with Corey's oxazaborolidine catalyst (*R*)-**9**[10] and borane in THF gave the alcohol (*S*)-**7** in 72% yield with 78% ee. The configuration was assigned according to Corey's model for CBS reductions.[11] In the same manner, reduction of ketone **8** with the enantiomeric catalyst (*S*)-**9** yielded the alcohol (*R*)-**7** with 81% ee. Experiments to reduce ketone **8** with (-)*B*-chlorodiisopino-

campheylborane [(-)-DIP-Chloride][12] or the oxazaborolidine derived from (1*S*,2*R*)-2-amino-1,2-diphenylethanol[13] and $BH_3 \times Me_2S$ were less satisfactory. Ozonolysis of either (*S*)-**7** or (*R*)-**7** afforded the aldehydes (*S*)-**3** or (*R*)-**3** in optically enriched form.

In order to confirm their absolute configuration, the synthetic hydroxyaldehydes (*S*)-**3** and (*R*)-**3** were esterified with Mosher's (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetyl (MTPA) chloride[14] in the presence of DMAP and scandium(III)-triflate.[15] The configuration of the resulting MTPA esters (*S,S*)- and (*S,R*)-**10** was determined by applying Kakisawa's high field ^1H NMR variant of Mosher's method.[16] Molecular mechanics calculations[17] for the diastereomeric esters **10** yielded minimum energy conformers in accord with the configurational correlation model proposed by Mosher[18] (Figure 1). The characteristic shielding effects $\Delta\delta$ of the phenyl ring in the MTPA esters (*S,S*)-**10** and (*S,R*)-**10** were determined after complete assignment of the protons by $^1H,^1H$-COSY experiments.

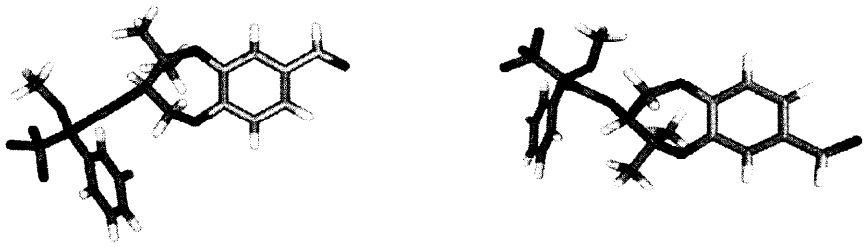
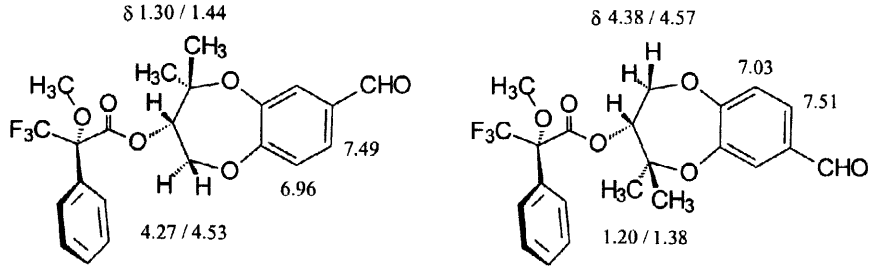


Figure 1. Minimum energy conformers of the MTPA esters (*S,S*)-**10** and (*S,R*)-**10** found using molecular mechanics

The methyl groups 4'-CH_3 and 5'-CH_3 show positive values for $\Delta\delta = \delta(S,S) - \delta(S,R)$ [+0.10 and +0.06, respectively], whereas the $\Delta\delta$ values for the diastereotopic protons at the oxepin ring are negative [1a-H: −0.11, 1b-H: −0.04]. The absolute stereochemistry of the synthetic aldehydes can therefore be assigned as given in the formulas (*S,S*)-**10** and (*S,R*)-**10** (Figure 2).

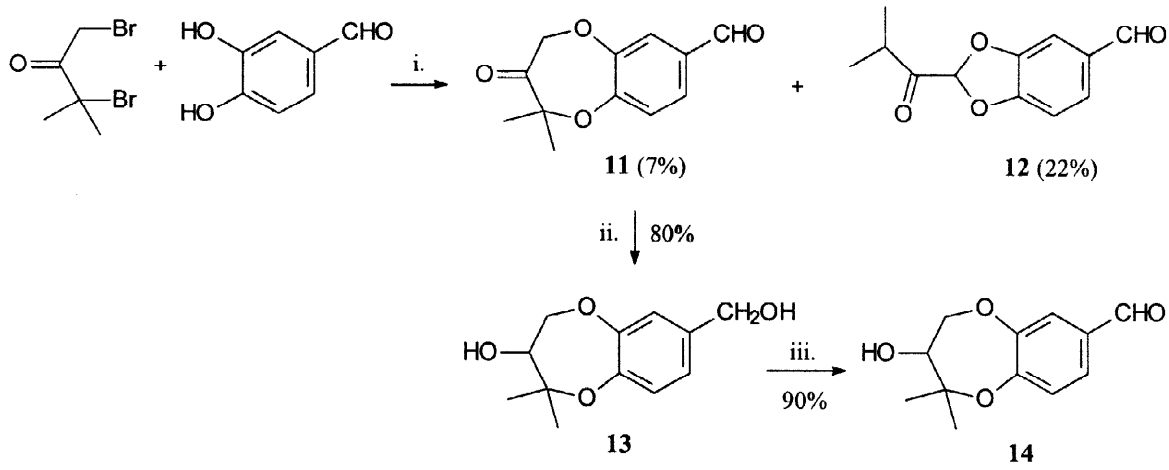


(*S,S*)-**10** (*S,R*)-**10**

Figure 2. MTPA esters (*S,S*)-**10** and (*S,R*)-**10**

The aldehyde **3** obtained from the ozonolysis of strobilurin I (**1**) was esterified with (*R*)-MTPA chloride.[14] GC/MS comparison with the synthetic MTPA esters (*S,S*)-**10** and (*S,R*)-**10** proved the identity of the MTPA ester derived from the ozonolysis product with (*S,S*)-**10**. Strobilurin I has therefore the structure and absolute configuration given in formula **1**. In addition, the trimethylsilyl derivative of the degradation product **3** and that of the regioisomeric aldehyde **14** were non-identical by GC/MS comparison, which excludes the inverted mode of oxepin ring attachment.

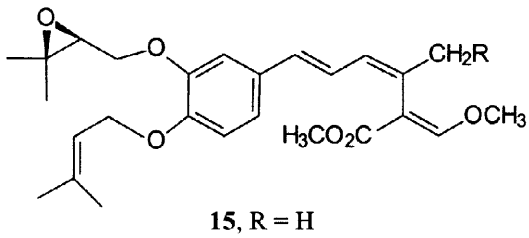
The synthesis of the racemic aldehyde **14** started from 3,4-dihydroxybenzaldehyde, which on base catalysed condensation with 1,3-dibromo-3-methylbutan-2-one under phase transfer conditions afforded a mixture of benzodioxepinone **11** (7%) and the isomeric benzodioxol derivative **12** (22%) (Scheme 2). The structure of **11** was rigorously proven by a single crystal X-ray analysis.[19] Reduction of the oxoaldehyde **11** with $NaBH_4/CeCl_3$ and selective reoxidation of the resulting diol **13** with manganese dioxide yielded the aldehyde **14**.



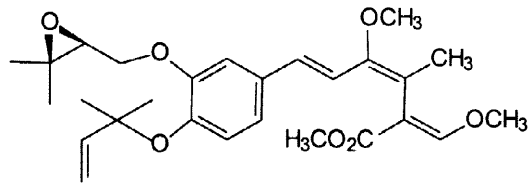
Scheme 2. Synthesis of aldehyde **16**. Reagents and conditions: (i). $Bn(n\text{-}Bu)_3N^+Br^-$ (0.2 equ), NaOH (2 equ), CH_2Cl_2/H_2O, 16 h; chromatographic separation on SiO_2. (ii). $NaBH_4$, $CeCl_3 \times 6\ H_2O$, EtOH–H_2O (7:5), -5 °C. (iii). MnO_2, CH_2Cl_2.

Structural revision of strobilurin D and hydroxystrobilurin D

The co-occurrence of strobilurin I (**1**) with strobilurin D indicates a close biogenetic relationship and prompted us to reinvestigate the epoxide structure **15** previously assigned to this compound.[3] Short treatment of



15, R = H
16, R = OH



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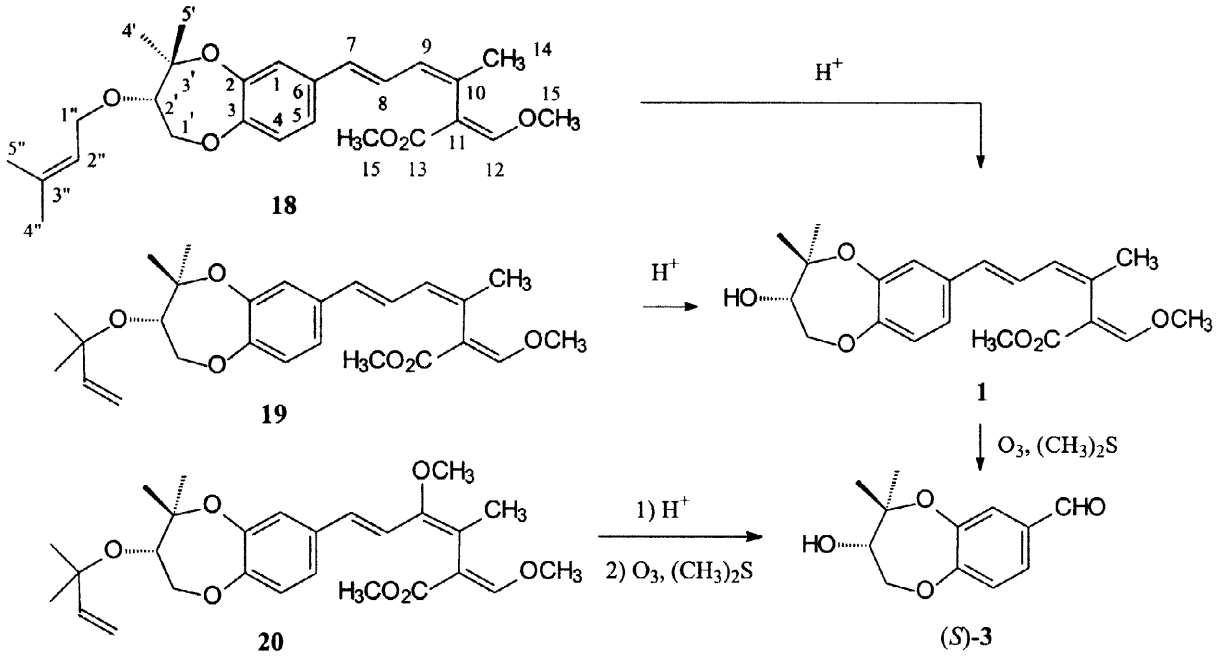
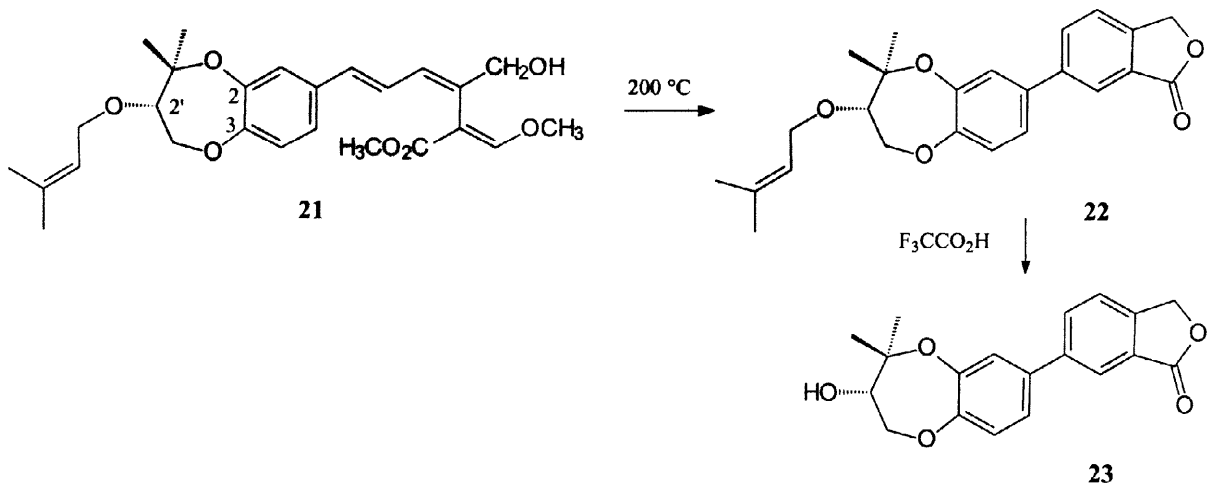
strobilurin D with trifluoroacetic acid removed the dimethylallyl residue and yielded a product with the same spectral data and optical rotation as natural strobilurin I (**1**) (Scheme 3). Ozonolysis of the dealkylation product



Scheme 3. Degradation of the benzodioxepin type strobilurins **1**, **18**, **19** and **20** to aldehyde (*S*)-**3**

afforded aldehyde (*S*)-**3** whose optical integrity was determined by GC/MS of the (*S,S*)-MTPA ester as described above. This finding excludes formula **15** previously assigned to strobilurin D[3] and proves the benzodioxepin structure and absolute stereochemistry given in formula **18**. The structural correction has already been suggested by Nicholas et al.[20] during their work on methoxystrobilurin K. Fredenhagen et al.[21] have previously assigned structure **18** (without stereochemistry) to strobilurin G isolated from cultures of the ascomycete *Bolinea lutea*. Since the NMR data and optical rotations of strobilurin D and G show close correspondence, both compounds must be identical.

The revision of the structure for strobilurin D affects also that of hydroxystrobilurin D[5] whose former formula **16** has to be corrected in **21**. We had previously observed the formation of a biphenyl derivative **22** on thermolysis of this antibiotic.[5] Treatment of this product with acid removed the dimethylallyl residue and yielded benzodioxepin **23**. In light of the revised structure **21**, the thermolysis of hydroxystrobilurin D has to be reformulated as given in Scheme 4. **23** exhibits a coupling pattern for C-2 and C-3 in the ^{1}H-coupled ^{13}C NMR that agrees with that of aldehyde **3** and differs from that of aldehyde **14**. The correspondence of the optical rotation of hydroxystrobilurin D ($[\alpha]_D^{22} = +22$, $CHCl_3$) with that of strobilurin D ($[\alpha]_D^{20} = +23.8$, MeOH) indicates the (2'-*S*)-configuration for both compounds.



Scheme 4. Reformulation of the thermolysis of hydroxystrobilurin D (**17**)[5]

Strobilurin K and structural revision of 9-methoxystrobilurin K

Cultures of *Mycena tintinnabulum* (Fr.) Quél. sensu Schroeter produce strobilurin D (= strobilurin G) (**18**) as well as an isomer, named strobilurin K. The only difference in the NMR spectra of both compounds is the presence of signals for a reversed 1,1-dimethylallyl residue in the spectrum of strobilurin K (Table 2) instead of the usual 3,3-dimethylallyl signals, which leads to formula **19**. Shape and position of the C-2 and C-3 signals in the ^{1}H-coupled ^{13}C NMR spectrum of **19** agree well with those of all the other strobilurins described in this publication. Structure and absolute configuration of **19** were again established by degradation to strobilurin I (**1**), followed by ozonolysis to aldehyde (*S*)-**3** and GC/MS comparison of the Mosher ester derived therefrom.

A comparison of the NMR data of **19** with those of 9-methoxystrobilurin K (Table 2) showed close agreement. For the latter compound the isomeric epoxide formula **17** had been assigned previously.[22] Recently, Nicholas et al.[20] isolated the same compound from a New Zealand species of *Favolaschia* and corrected structure **17** to **20** on the basis of NMR arguments.[23] Structure **20** including the absolute configuration was confirmed by the degradation of 9-methoxystrobilurin K to aldehyde (*S*)-**3** (Scheme 3), which was identified by GC/MS analysis of the corresponding MTPA ester.

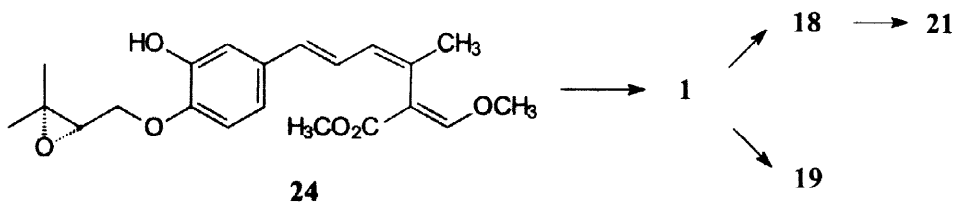
Table 2. ^{1}H and ^{13}C NMR spectra of strobilurin K (**19**) in [D_6]acetone and 9-methoxystrobilurin K (**20**) in CD_3OD

	19				**20**			
position	δ_C	$^{n}J_{CH}$ [Hz]	δ_H		δ_C	$^{n}J_{CH}$ [Hz]	δ_H	
1	122.20	Ddd (157.2, 7.3, 5.4, 1.4)	6.90	d	122.64	Ddd (157.4, 7.4, 5.3)	6.92	d
2	147.40	ddd (7.1, 4.2, 1.4)			147.84	Ddd (7.1, 4.3, 1.4)		
3	151.57	m			152.28	M		
4	120.89	Ddd (158.7, 1.6, 1.0)	6.77	d	121.35#	Ddd (158.3, 1.8, 0.9)	6.84	d
5	122.73	Dddbr (158.2, 7.6, 5.0)	6.96	dd	123.49	Dddd (157.4, 7.8, 4.8, 1.9)	6.99	dd
6	134.13	ddd (9.1, 5.6, 2.1)			134.18	ddd (9.0, 5.7, 2.4)		
7	130.53	Dm (150.6)	6.36	d	128.00	Dddd (152.6, 4.7, 4.7, 1.9)	6.58	d
8	126.90	Dd (149.6, 1.5)	6.53	dd	121.39#	Dd (150.7, 1.4)	6.41	d
9	130.40	Dm (151.6)	6.13	d	153.98	m		
10	131.77	m			119.42	m		
11	111.09	m			111.46	m		
12	159.96	Dq (181.0, 5.1)	7.44	s	161.42	Dq (181.0, 5.1)	7.53	s
13	167.68	m			169.76	m		
14	23.87	Qd (127.6, 6.6)	1.89	s	16.50	Q (127.9)	1.89	s
15	62.06	Qd (145.5, 6.6)	3.86	s	62.40	Qd (145.4, 6.2)	3.88	s
16	51.34	Q (146.5)	3.64	s	52.00	Q (146.4)	3.75	s
17					59.81	Q (143.1)	3.67	s
1'	72.21	T (146.0)	a: 3.94	dd	72.58	T (145.9)	a: 4.03	dd
			b: 4.19	dd			b: 4.24	dd
2'	76.27	Dm (140.4)	3.70	dd	76.94	Dm (140.6)	3.73	dd
3'	82.14	m			82.73	m		
4'	21.93	Qm (127.1)	1.17	s	22.62	Qm (127.3)	1.26	s
5'	28.47	Qm (127.2)	1.38	s	28.21	Qm (126.8)	1.43	s
1"	76.77	m			77.41	m		
2"	144.77	Dm (154.1)	5.94	dd	145.03	Dm (154.0)	5.98	dd
3"	114.59	DD (157.9, 155.2)	a: 5.22	d	114.94	DD (157.6, 155.1)	a: 5.27	d
			b: 5.15	d			b: 5.21	d
4"	27.03#	Qm (126.5)	1.30#	s	26.74+	Qm (126.5)	1.36	s
5"	26.58#	Qquin (126.5)	1.31#	s	27.14+	Qm (126.7)	1.36	s

19: *J*[Hz]: 1–5 = 1.8; 4–5 = 8.3; 7–8 = 15.6; 8–9 = 10.8; 1'a–1'b = 12.3; 1'a–2' = 7.9; 1'b–2 = 3.3; 2"–3"a = 17.6; 2"–3"b = 10.8.
20: *J*[Hz]: 1–5 = 1.9; 4–5 = 8.3; 7–8 = 15.9; 1'a–1'b = 12.4; 1'a–2' = 7.4; 1'b–2 = 3.1; 2"–3"a = 17.6; 2"–3"b = 10.9.
or +: signals may be interchanged.

Conclusions

As a result of the investigations described in this paper the open chain epoxide structures previously assigned to strobilurin D (= strobilurin G), hydroxystrobilurin D, and 9-methoxystrobilurin K are wrong and have to be replaced by the benzodioxepin structures **18**, **21** and **20**, respectively. In all cases the regiochemistry of the attachment of the 1,4-dioxepin ring and the absolute configuration at C-2' are the same as in strobilurin I (**1**), which may function as biogenetic precursor of the ether derivatives **18** and **19**. It may be suggested that strobilurin I arises in turn by an enzyme catalysed cyclisation of epoxide **24** or oxidative cyclisation of the corresponding dimethylallyl ether (Scheme 5).



Scheme 5. Proposal for the biosynthesis of the benzodioxepin type strobilurins from epoxide **24**

Biological activities

Strobilurin I (**1**) and strobilurin K (**19**) exhibit strong antifungal activities which closely resemble those of the other naturally occurring (*E*)-ß-methoxyacrylates.[3,25-27] The new antibiotics inhibit a broad spectrum of different fungi at concentrations of 0.1-1 µg/disc (Table 3). Both compounds are potent inhibitors of eucaryotic respiration. Oxygen uptake of *Penicillium notatum* is blocked 20% by 0.1 µg/ml of strobilurin I (0.27 µM) and 75% by 0.1 µg/ml of strobilurin K (0.23 µM). 1 µg/ml of both strobilurins completely inhibit fungal respiration. No antibacterial activities could be detected. As described for other strobilurins,[3,26,27] **1** and **19** exhibit cytostatic effects on HeLa S3 cells at 0.4-2 µg/ml (1.07-5.35 µM) and 0.05 µg/ml (0.11 µM) respectively.

Table 3. Antifungal activities of strobilurin I (**1**) and K (**19**) in the plate diffusion assay.

Test organism	Diameter of inhibition zone [mm]			
	Strobilurin I (**1**)		Strobilurin K (**19**)	
	µg/paper disc (∅ 6 mm)			
	0.1	1	0.1	1
Absidia glauca (+)	-	9 i	-	10
Absidia glauca (-)	-	8 i	9	14
Alternaria porri	12 i	n.t.	11 i	25 i
Aspergillus ochraceus	-	14 i	-	15
Botrytis cinerea	-	26 i	-	13
Curvularia lunata	-	13 i	9	15
Epicoccum purpurascens	-	10 i	-	10 i
Fusarium fujikuroi	-	-	-	-
Fusarium oxysporum	-	10 i	-	-
Mucor miehei	-	17 i	11	20
Nematospora coryli	-	8 i	-	18 i
Paecilomyces variotii	-	8 i	-	10 i
Penicillium notatum	9 i	26 i	10	18
Phoma clematidina	22 i	n.t.	13 i	20 i
Rhodotorula glutinis	-	9	10 i	19 i
Saccharomyces cerevisiae is 1	-	8 i	-	11
Ustilago nuda	-	-	9	15
Zygorhynchus moelleri	-	17 i	-	13 i

- = no inhibition zone, i = incomplete inhibition zone, n.t. = not tested

EXPERIMENTAL SECTION

General. Melting points (uncorrected): Reichert Thermovar. - Optical rotations: Perkin-Elmer 214. - UV/Vis and CD: Instruments S. A. Jobin Yvon CD-6-Dichrograph. - IR: Bruker FTIR spectrophotometer IFS 45 or IFS 48. - NMR: Bruker AMX 600 and ARX 300 with solvent peak as internal reference ($CDCl_3$: δ_H 7.24, δ_C 77.0; CD_3OD: δ_H 3.35, δ_C 49.0; [D_6]acetone: δ_H 2.04, δ_C 29.8). - Long range ^1H-^{13}C connectivities were determined by inverse experiments using gradient pulses optimised for a coupling constant of ~7 Hz. - TLC: Silica gel Merck G plates. - Column chromatography: Silica gel Merck 60. - Preparative MPLC and HPLC were carried out on a Labomatic MD 80/100 equipped with a LiChroprep Si 60, 0.025-0.04 mm column 331 mm × 26 mm, respectively a Merck LiChrosorb DIOL 7μm column 250 mm × 25 mm. - EI MS, HR-EI MS and gas chromatography/mass spectrometry (GC/MS) were performed on a Finnigan MAT 95 double focusing mass spectrometer, equipped with an EI ion source operated at 70 eV. For GC/MS a Varian GC 3400 gas chromatograph with a fused silica DB-5ms capillary column (30 m × 0.25 mm, coated with a 0.1 μm layer of liquid phase) and He as carrier gas was used for sample separation. The injector temperature was kept at 300 °C, injection volumes were 0.2–0.4 μL of a 1–2% (m/v) solution. Temperature programme: 2 min isothermal at 200 °C, then 5 K/min up to 300 °C, finally 10 min isothermal at 300 °C. Kovats indices were determined by co-injection of a 0.2 μL sample of a standard mixture of saturated straight chain alkanes (C_{10}–C_{36}).

Producing organisms, culture conditions: Mycelial cultures of *Agaricus* sp. strain 89139 and *Mycena tintinnabulum* strain 96001 were derived from spore prints of fruiting bodies. The strains are deposited in the culture collection of the LB Biotechnologie, University of Kaiserslautern. For maintenance on agar slants the fungi were grown on YMG medium composed of (g/L): yeast extract 4, malt extract 10, glucose 4, and agar 15, pH 5.5.

Biological tests: The antimicrobial spectra were determined in the plate diffusion assay and tests for cytotoxic activity were carried out as described previously.[26] The inhibition of respiration was tested as described by Weber et al.[27]

Fermentation of *Agaricus* sp. 89139 and isolation of strobilurin I (1). Fermentations were carried out in a 20 L Biostat U fermentor equipped with a MFCS system (B. Braun Biotech) at 24 °C with an aeration rate of 3 L air/min and agitation (130 rpm). The fermentation medium was composed of (g/L): glucose 10.5, yeast extract 6, KH_2PO_4 0.3, Hoagland A-Z solution 1 mL. The pH was adjusted to 5.5 prior to sterilisation. 250 mL of a well grown culture in YMG medium were used as inoculum. During fermentation antifungal activity was measured in the agar plate diffusion assay with *Mucor miehei* as test organism. After 6–11 days of fermentation, the culture fluid (18 L) was separated from the mycelia. **1** was removed from the culture fluid by adsorption to HP21 resin (Mitsubishi) and eluted with 2 L of acetone. After evaporation of the acetone, the compounds were extracted from the residual aqueous phase with three times the equal volume of EtOAc. The crude extract (1.25 g) obtained after removal of the solvent was purified by MPLC on silica gel (LiChroprep Si 60, 0.025-0.04 mm; column 331 × 26 mm; elution with cyclohexane/EtOAc, 7:3) yielding 28 mg of an enriched product. Further purification of **1** was achieved by preparative HPLC on Merck LiChrosorb DIOL (7 μm, column 250 × 25 mm, elution with cyclohexane/*t*-BuOMe, 1:1). Yield: 3 mg.

Strobilurin I (1): pale yellow oil. - TLC: R_f = 0.49 (toluene/acetone, 7:3). - $[\alpha]_D^{20}$ = +20 (*c* = 0.14, MeOH). - UV/Vis (MeOH): λ_{max} (lg ε) = 314 nm (sh, 4.742), 302 (4.784), 290 (sh, 4.740), 230 (4.738). - IR (KBr): $\tilde{\nu}$ = 3440 cm^{-1} (br, m), 2980 (m), 2940 (m), 1710 (st), 1630 (st), 1570 (w), 1500 (st), 1420 (m), 1395 (w), 1370 (w), 1265 (st), 1240 (st), 1190 (w), 1145 (st), 1120 (st), 1070 (st), 1030 (w), 980 (m), 905 (w), 815 (w), 770 (w). - ^{1}H NMR and ^{13}C NMR see Table 1 - EI MS (DE 180 °C): *m/z* (%) = 375 (19), 374.1747 (374.1729 calcd for $C_{21}H_{26}O_6$, 100) [M^+], 342 (9), 315 (5), 283 (5), 258 (11), 257 (5), 238 (11), 237 (94), 153 (26), 123 (8), 75 (29).

***O*-Acetylstrobilurin I (2):** To a solution of **1** (3 mg) in CH_2Cl_2 (3 mL) pyridine (1 mL), acetic anhydride (0.5 mL), and a catalytic amount of DMAP were added. The mixture was kept for 40 h at 25 °C and then was washed successively with 2.5% aqueous citric acid and water. After evaporation of the dried (Na_2SO_4) organic layer, a red oil was obtained, which was purified by HPLC on RP 18 to yield 2.1 mg of **2** as a colourless oil. - TLC: R_f = 0.96 (toluene/acetone, 7:3). - $[\alpha]_D^{23}$ = +76 (*c* = 0.10, MeOH). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 3 H; 4- or 5'-H), 1.36 (s, 3 H; 4'- or 5'-H), 1.93 (d, *J* = 1.3 Hz, 3 H, 14-H), 2.15 (s, 3 H; CH_3CO), 3.71 (s, 3 H; 16-H), 3.82 (s, 3 H; 15-H), 4.11 (dd, *J* = 13.0 and 5.0 Hz, 1 H; 1'a-H), 4.21 (dd, *J* = 13.0 and 2.7 Hz, 1 H; 1'b-H), 5.00 (dd, *J* = 5.0 and 2.7 Hz, 1 H; 2'-H), 6.20 (dq, *J* = 10.5 and 1.3 Hz, 1 H; 9-H), 6.35 (d, *J* = 15.5 Hz, 1 H; 7-H), 6.48 (dd, *J* = 15.5 and 10.5 Hz, 1 H; 8-H), 6.84 (d, *J* = 7.5 Hz, 1 H; 4-H), 6.93 (m, 1 H; 5-H), 6.94 (m, 1 H; 1-H), 7.40 (s, 1 H; 12-H). - EI MS (DE 80 °C): *m/z* (%) = 417 (26), 416.1820 (416.1835 calcd. for $C_{23}H_{28}O_7$, 100) [M^+], 384 (8), 357 (5), 325 (5), 300 (7), 279 (66), 153 (4), 127 (10).

Ethyl 3-[3-Hydroxy-4-(3-methyl-but-2-enyloxy)phenyl]acrylate (5): A solution of **4** (10.4 g, 50.0 mmol) in DMF (150 mL) was cooled to 0 °C and 3,3-dimethylallyl bromide (6.0 mL, 50.0 mmol) and K_2CO_3 (6.91 g, 50 mmol) were added. After 16 h, 2 N HCl (100 mL) was added and the reaction mixture extracted with EtOAc (3 ×). The organic layers were washed with water (2 ×) and dried ($MgSO_4$). After evaporation, an orange oil was obtained. Chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) yielded 8.79 g (64%) of pure **5**. Colourless solid. - M.p. 79° C. - TLC: R_f = 0.65 (petroleum ether/EtOAc, 3:1). - IR (KBr): $\tilde{\nu}$ = 3405 cm^{-1} (m, br), 3063 (w), 3026 (w), 2994 (m), 2981 (m), 2939 (w), 2913 (w), 1697 (st), 1634 (st), 1610 (st), 1580 (m), 1510 (st), 1460 (m), 1388 (m), 1373 (m), 1317 (st), 1286 (st), 1268 (st), 1231 (m), 1187 (st), 1161 (m), 1131 (st), 1038 (w), 996 (st), 979 (st), 924 (w), 856 (m), 806 (m), 784 (w), 758 (w), 718 (w), 633 (w), 612 (w), 579 (w). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, *J* = 7.2 Hz, 3 H), 1.74 (d, *J* = 1.4 Hz, 3 H), 1.80 (d, *J* = 1.4 Hz, 3 H), 4.24 (q, *J* = 7.2 Hz, 2 H), 4.60 (d, *J* = 6.8 Hz, 2 H), 5.47 (tqq, *J* = 6.8, 1.4, 1.4 Hz, 1 H), 5.76 (s, 1 H, OH), 6.27 (d, *J* = 15.9 Hz, 1 H), 6.83 (d, *J* = 8.4 Hz, 1 H), 6.99 (dd, *J* = 8.4 and 2.1 Hz, 1 H), 7.13 (d, *J* = 2.1 Hz, 1 H), 7.58 (d, *J* = 15.9 Hz, 1 H).- ^{13}C NMR (300 MHz, $CDCl_3$): δ = 14.42, 18.32, 25.86, 60.42, 65.83, 111.73, 113.02, 116.25, 118.84, 121,74, 128.02, 139.45, 144.55, 146.17, 147.85, 167.40. - MS (EI): *m/z* (%) = 276 (4) [M^+], 208 (100), 180 (15), 163 (44), 136 (14), 69 (37). Anal. Calcd. for $C_{16}H_{20}O_4$: C, 69.55; H, 7.29. Found: C, 69.25; H, 7.56.

Ethyl 3-[4-(3,3-Dimethyl-oxiranylmethoxy)-3-hydroxyphenyl]acrylate (6): A solution of **5** (11.1 g, 40.0 mmol) in CH_2Cl_2 (120 mL) was cooled to 0 °C and 75% *m*CPBA (10.4 g, 60.0 mmol) was added. After 6 h, the solvent was evaporated, the reaction mixture dissolved in EtOAc and washed with saturated $NaHCO_3$ (2 ×) and water. After evaporation of the dried ($MgSO_4$) organic layers, an orange oil was obtained that was purified by chromatography on a silica gel column (petroleum ether/EtOAc, 3:1). Crystallisation from petroleum ether/EtOAc afforded 11.3 g (96%) of pure **6**. Colourless solid. - M.p. 74 °C. - TLC: R_f = 0.22

(petroleum ether/EtOAc, 3:1). - IR (KBr): $\tilde{\nu}$ = 3422 cm^{-1} (m, br), 2986 (m), 2960 (m), 2928 (m), 1699 (st), 1635 (st), 1612 (st), 1582 (st), 1513 (st), 1450 (m), 1373 (m), 1314 (st), 1288 (st), 1268 (st), 1186 (st), 1162 (st), 1127 (st), 1044 (m), 1018 (m), 982 (m), 862 (m), 838 (w), 802 (m), 611 (w), 578 (w), 556 (m). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, *J* = 7.1 Hz, 3 H), 1.37 (s, 3 H), 1.40 (s, 3 H), 3.16 (dd, *J* = 6.3 and 3.9 Hz, 1 H), 4.11 (dd, *J* = 11.2 and 6.3 Hz, 1 H), 4.25 (q, *J* = 7.1 Hz, 2 H), 4.33 (dd, *J* = 11.2 and 3.9 Hz, 1 H), 5.94 (s, 1 H, OH), 6.28 (d, *J* = 15.9 Hz, 1 H), 6.88 (d, *J* = 8.3 Hz, 1 H), 7.02 (dd, *J* = 8.3 and 2.0 Hz, 1 H), 7.14 (d, *J* = 2.0 Hz, 1 H), 7.58 (d, *J* = 15.9 Hz, 1 H). - ^{13}C NMR (75.6 MHz, $CDCl_3$): δ = 14.41, 19.12, 24.66, 58.55, 60.46, 61.06, 68.45, 112.22, 113.67, 116.78, 121.62, 128.89, 144.29, 146.21, 147.49, 167.29. - MS (EI): *m/z* (%) = 292 (100) [M^+], 247 (18), 208 (71), 180 (12), 163 (25), 85 (71), 57 (13). - Anal. Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 66.04; H, 7.10.

Ethyl 3-(3-Hydroxy-4,4-dimethyl-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)acrylate [(*R,S*)-7]: To a stirred suspension of $La(OTf)_3$ (2.93 g, 5.0 mmol) in CH_2Cl_2 (20 mL), water (18.0 mL, 1 mol) and a solution of **6** (5.85 g, 20 mmol) in CH_2Cl_2 (100 mL) were added. After 7 days stirring at room temperature, the reaction mixture was put into sat. aqueous $NaHCO_3$ (20 mL), and the precipitating solid was dissolved on addition of 2 N HCl (30 mL). The aqueous layer was extracted with EtOAc (2 ×) and the combined organic layers were dried over $MgSO_4$. After evaporation, an orange oil was obtained. Chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) yielded 1.15 g (20%) (*R,S*)-**7**. Colourless oil. - TLC: R_f = 0.24 (petroleum ether/EtOAc, 3:1). - IR (KBr): $\tilde{\nu}$= 3464 cm^{-1} (m, br), 2979 (st), 2905 (m), 1709 (st), 1636 (st), 1604 (m), 1572 (m), 1503 (st), 1455 (m), 1423 (m), 1368 (st), 1321 (st), 1265 (st), 1178 (st), 1159 (st), 1116 (st), 1096 (st), 1067 (st), 1034 (st), 983 (st), 906 (m), 827 (m), 813 (m), 733 (m), 677 (w), 646 (w), 593 (w). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (s, 3 H), 1.33 (t, *J* = 7.2 Hz, 3 H), 1.52 (s, 3 H), 2.85 (d, *J* = 10.8 Hz, 1 H, OH), 3.60-3.64 (m, 1 H), 4.14 (dd, *J* = 12.6 and 7.3 Hz, 1 H), 4.19 (dd, *J* = 12.6 and 4.2 Hz, 1 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 6.32 (d, *J* = 16.0 Hz, 1 H), 7.00 (d, *J* = 7.8 Hz, 1 H), 7.13-7.18 (m, 2 H), 7.57 (d, *J* = 16.0 Hz, 1 H). - ^{13}C NMR (75.6 MHz, $CDCl_3$): δ = 14.38, 24.27, 25.29, 60.53, 71.12, 75.42, 80.72, 117.72, 121.01, 123.67, 124.66, 131.06, 143.59, 147.98, 153.91, 167.10. - MS (EI): *m/z* (%) = 292.1303 (292.1311 calcd. for $C_{16}H_{20}O_5$, 100) [M^+], 248 (30), 247 (17), 234 (12), 233 (34), 208 (81), 205 (12), 180 (18), 163 (36), 147 (11), 136 (17), 134 (10). - Anal. Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.57; H 7.11.

Ethyl 3-(4,4-Dimethyl-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)acrylate (8): To a suspension of pyridinium chlorochromate (0.39 g, 1.8 mmol) in CH_2Cl_2 (3 mL) a solution of **7** (0.34 g, 1.17 mmol) in CH_2Cl_2 (5 mL) was added. After 24 h at room temperature, the reaction mixture was diluted with anhydrous Et_2O (20 mL), filtered and the black solid washed with ether (2 ×). Evaporation of the solvent and chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) gave a colourless oil, which was further purified by crystallisation from petroleum ether/EtOAc to yield 0.20 g (59%) of **8**. Colourless needles. - M.p. 78°C - TLC: R_f = 0.75 (petroleum ether/EtOAc, 3:1). - IR (KBr): $\tilde{\nu}$ = 3437 cm^{-1} (m), 3058 (w), 2987 (m), 2941 (w), 2903 (w), 1727 (st), 1718 (st), 1634 (st), 1606 (w), 1576 (m), 1504 (st), 1465 (w), 1431 (w), 1383 (w), 1365 (w), 1348 (w), 1298 (m), 1283 (st), 1266 (st), 1165 (st), 1130 (w), 1114 (w), 1032 (m), 998 (m), 945 (w), 918 (w), 908 (w), 859 (w), 848 (w), 820 (m), 799 (w), 777 (w), 756 (w), 706 (w), 652 (w), 598 (w), 575 (w), 538 (w), 479 (w), 423 (w). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, *J* = 7.2 Hz, 3 H), 1.49 (s, 6 H), 4.24 (q, *J* = 7.2 Hz, 2 H), 4.79 (s, 2 H), 6.31 (d, *J* = 16.0 Hz, 1 H), 6.93 (d, *J* = 8.2 Hz, 1 H), 7.15 (dd, *J* = 8.2 and 2.1 Hz, 1 H), 7.18 (d, *J* = 2.1 Hz, 1 H), 7.56 (d, *J* = 16.0 Hz, 1 H). - ^{13}C NMR (75.6 MHz, $CDCl_3$): δ = 14.38,

24.63 (2 ×), 60.52, 74.67, 87.83, 117.57, 120.23, 123.01, 124.29, 130.15, 143.34, 144.70, 150.11, 167.04, 207.14. - MS (EI): *m/z* (%) = 290 (82) [M^+], 262 (85), 245 (18), 219 (100), 207 (34), 179 (22), 161 (14), 133 (12), 89 (5), 69 (11), 55 (11). - Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.20; H, 6.25. Found: C, 66.16; H 6.19.

Enantioselective reduction of ketone 8 to (*S*)-7. To a 0.13 M solution of (*R*)-tetrahydro-1-methyl-3,3-diphenyl-1*H*, 3*H*-pyrrolo[1,2-*c*][1,3,2]oxazaborole [(*R*)-**9**][10] (0.7 mL, 0.09 mmol) in toluene at 0 °C were added THF (2 mL) and $BH_3 \times Me_2S$ (0.16 mL, 1.69 mmol). After 5 min, a solution of **8** (0.83 g, 2.86 mmol) in THF (10 mL) was added dropwise. After another 30 min, the reaction mixture was quenched with water and extracted with EtOAc (2 ×). The organic layers were dried over $MgSO_4$. Evaporation and chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) yielded 0.60 g (72%) of (*S*)-**7** (78% ee). The enantiomeric excess was determined by ozonolysis to aldehyde **3** and GC/MS of the corresponding (*R*)-MTPA-ester as described below.

Enantioselective reduction of ketone 8 to (*R*)-7. From **8** (0.16 g, 0.54 mmol) and (*S*)-**9**[10] (0.12 mL of 0.13 M solution, 0.02 mmol) as described above. Yield 0.12 g (73%), 81% ee.

(*S*)-3-Hydroxy-4,4-dimethyl-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-carbaldehyde [(*S*)-3]: A solution of (*S*)-**7** (14 mg, 48 μmol, 78% ee) in CH_2Cl_2 (10 ml) was cooled to -78 °C and saturated with ozone. After 5 min, dimethyl sulfide (1 mL) was added and the solution allowed to warm to room temperature. Evaporation and chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) yielded 10 mg (94%) of (*S*)-**3** (78% ee). Colourless oil. - TLC: R_f = 0.57 (petroleum ether/EtOAc, 1:1). - $[\alpha]_D^{20}$ = +39.7 (*c* = 1 in $CHCl_3$). - IR (KBr): $\tilde{\nu}$ = 3444 cm^{-1} (m, br), 3063 (w), 2978 (st), 2938 (st), 2732 (m), 1694 (st), 1600 (st), 1584 (s), 1502 (st), 1430 (st), 1392 (st), 1370 (st), 1278 (st), 1154 (st), 1099 (st), 1066 (st), 1019 (st), 986 (st), 940 (m), 904 (m), 848 (m), 830 (m), 784 (m), 734 (m), 678 (m), 612 (m). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (s, 3 H), 1.50 (s, 3 H), 3.65-3.71 (m, 1 H), 4.20 (dd, *J* = 12.6 and 1.7 Hz, 1 H), 4.23 (dd, *J* = 12.6 and 4.1 Hz, 1 H), 4.56 (s, 1 H, OH), 7.12 (d, *J* = 8.1 Hz, 1 H), 7.50 (d, *J* = 2.0 Hz, 1 H), 7.53 (dd, *J* = 8.1 and 2.0 Hz, 1 H), 9.87 (s, 1 H). - ^{13}C NMR (75.6 MHz, $CDCl_3$): δ = 24.56, 25.01, 71.35, 75.32, 81.15, 121.19, 124.50, 125.37, 133.13, 148.15, 157.49, 190.76. - MS (EI): *m/z* (%) = 222 (100) [M^+], 178 (27), 164 (20), 163 (63), 152 (9), 149 (24), 138 (67), 137 (46), 123 (11), 85 (12), 71 (19), 43 (18), 41 (10).

Aldehyde (*R*)-3: From (*R*)-**7** (14 mg, 48 μmol, 81% ee) as described for the (*S*)-enantiomer. Yield 10 mg (94%), 81% ee. - $[\alpha]_D^{20}$ = –34.3 (*c* = 1 in $CHCl_3$).

Reaction of 3,4-dihydroxybenzaldehyde with 1,3-dibromo-3-methylbutan-2-one. To a suspension of 3,4-dihydroxybenzaldehyde (4.14 g, 30.0 mmol) in CH_2Cl_2 (80 ml) were added sequentially 1,3-dibromo-3-methylbutan-2-one[28] (7.32 g, 30 mmol), water (10 mL), and benzyl(tri-*n*-butyl)ammonium bromide (1.8 g, 5.0 mmol). Within 1 h, a solution of NaOH (2.4 g, 60 mmol) in water (40 mL) was added dropwise, and the solution was stirred for 16 h. After separation of the two phases, the aqueous layer was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$) and evaporated. The products **11** and **12** were separated by column chromatography on silica gel (petroleum ether/EtOAc, 3:1).

2,2-Dimethyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-carbaldehyde (11): Recrystallisation of the first fraction from EtOAc/petroleum ether yielded **11** (0.44 g, 7% yield) as colourless crystals. - M.p. 92 °C. - TLC: R_f = 0.67 (petroleum ether/EtOAc, 1:1). - IR (KBr): $\tilde{\nu}$ = 3437 cm^{-1} (w), 3032 (w), 2984 (w), 2861 (w),

2762 (w), 1730 (st), 1692 (st), 1601 (m), 1579 (w), 1500 (m), 1492 (m), 1460 (w), 1446 (m), 1440 (m), 1424 (w), 1400 (w), 1382 (w), 1364 (w), 1355 (w), 1318 (s), 1272 (st), 1248 (m), 1222 (w), 1189 (m), 1156 (m), 1131 (w), 1110 (m), 1034 (m), 947 (m), 935 (w), 901 (w), 868 (w), 827 (m), 789 (w), 741 (w), 719 (w), 674 (w), 627 (m), 594 (w), 536 (w). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.53 (s, 6 H), 4.77 (s, 2 H), 7.11 (d, J = 8.2 Hz, 1 H), 7.47 (d, J = 2.0 Hz, 1 H), 7.50 (dd, J = 8.2 and 2.0 Hz, 1 H), 9.85 (s, 1 H). - ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.14 (2C), 75.77, 88.53, 121.75, 124.20, 125.90, 133.11, 148.94, 150.85, 190.75, 206.48. - MS (EI): *m/z* (%) = 220 (44) [M^+], 192 (44) [$C_{11}H_{12}O_3^+$], 163 (11) [$C_9H_7O_3^+$], 149 (100) [$C_8H_5O_3^+$], 137 (13) [$C_7H_5O_3^+$], 69 (9), 55 (22). - Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.27; H 5.53.

2-Isobutyryl-benzo[1,3]dioxol-5-carbaldehyde (12): From the second fraction as a brownish oil (1.45 g, 22% yield). R_f = 0.63 (petroleum ether/EtOAc, 3:1). - IR (KBr): $\tilde{\nu}$ = 3365 cm^{-1} (w), 3078 (w), 2978 (m), 2937 (m), 2877 (w), 1736 (st), 1691 (st), 1604 (st), 1492 (st), 1448 (st), 1386 (m), 1352 (m), 1286 (st), 1259 (st), 1218 (m), 1159 (m), 1124 (st), 1102 (st), 1059 (st), 1025 (m), 996 (m), 908 (m), 816 (m), 786 (m), 626 (w), 584. - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.17, 1.18 (each d, J = 6.9 Hz, 6 H), 3.03 (sept, J = 6.9 Hz, 1 H), 6.32 (s, 1 H), 7.00 (d, J = 8.0 Hz, 1 H), 7.39 (d, J = 1.6 Hz, 1 H), 7.46 (dd, J = 8.0 and 1.6 Hz, 1 H), 9.84 (s, 1 H). - ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 18.24, 18.32, 36.39, 107.43, 107.75, 109.14, 129.29, 132.76, 148.44, 152.58, 190.49, 204.95. - MS (EI): *m/z* (%) = 220.0735 (220.0727 calcd. for $C_{12}H_{12}O_4$, 8) [M^+], 150 (8), 149 (100) [$C_8H_5O_3^+$].

3-Hydroxy-7-hydroxymethyl-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin (13): To a suspension of **11** (0.23 g, 1.04 mmol) in water (5 mL) and EtOH (7 mL) $CeCl_3 \times 6\ H_2O$ (0.19g, 0.52 mmol) was added. The mixture was stirred at -5 °C and treated with $NaBH_4$ (0.06 g, 1.56 mmol). After 10 min, the mixture was quenched with acetone and extracted with EtOAc (3 ×). The organic phases were washed with water, dried ($MgSO_4$) and evaporated. Chromatography on a silica gel column (petroleum ether/EtOAc, 1:1) yielded 15 as a colourless oil. Yield 0.19 g (80%). - R_f = 0.34 (petroleum ether/EtOAc, 1:1). - IR (KBr): $\tilde{\nu}$ = 3391 cm^{-1} (br), 2979 (st), 2936 (st), 1613 (w), 1582 (m), 1504 (st), 1456 (m), 1426 (st), 1385 (m), 1369 (m), 1262 (st), 1208 (m), 1160 (st), 1115 (st), 1092 (m), 1069 (st), 1035 (st), 946 (m), 919 (m), 880 (m), 859 (m), 829 (m), 762 (w), 738 (m), 680 (w), 603 (w). - ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.17, 1.47 (each s, 6 H), 2.79 (s, 2 OH), 3.54 (dd, J = 4.1 and 1.4 Hz, 1 H), 4.03 (dd, J = 12.6 and 1.4 Hz, 1 H), 4.12 (dd, J = 12.6 and 4.1 Hz, 1 H), 4.53 (s, 2 H), 6.91–6.96 (m, 3 H). - ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.89, 25.48, 64.43, 70.78, 75.42, 80.23, 120.24, 122.97, 124.28, 137.47, 147.09, 152.12. - MS (EI): *m/z* (%) = 224 (100) [M^+], 207 (7), 180 (22), 165 (71), 151 (20), 140 (83), 123 (27), 122 (47), 111 (18), 93 (16), 71 (12), 59 (2). - Anal. Calcd. for $C_{12}H_{16}O_4$: C, 65.27; H, 7.19. Found: C, 63.62; H 7.23.

3-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-carbaldehyde (14): **13** (0.32 g, 1.40 mmol) was dissolved in CH_2Cl_2 (20 mL) and stirred for 3 h with activated manganese dioxide (1.24 g, 14.0 mmol). The mixture was filtered through Celite and washed with acetone. Evaporation of the filtrate and chromatography on a silica gel column (petroleum ether/EtOAc, 1:1) yielded **14** as colourless oil (0.28 g, 90%). - TLC: R_f = 0.52 (petroleum ether/EtOAc, 1:1). - IR (KBr): $\tilde{\nu}$ = 3444 cm^{-1} (br), 3062 (m), 2980 (s), 2939 (m), 2732 (m), 2606 (m), 1694 (st), 1682 (st), 1601 (st), 1574 (st), 1495 (st), 1456 (m), 1431 (st), 1392 (st), 1370 (st), 1317 (st), 1275 (br), 1210 (m), 1145 (st), 1112 (st), 1068 (st), 1034m), 996 (m), 949 (m), 922 (m), 886 (m), 864 (m), 816 (m), 777 (m), 759 (m), 735 (m), 708 (w), 679 (w), 661 (m), 630 (m), 611 (m), 579 (m). - ^{1}H NMR

(300 MHz, $CDCl_3$): δ = 1.25 (s, 3 H), 1.53 (s, 3 H), 3.03 (s, 1 H, OH), 3.65 - 3.66 (m, 1 H), 4.14 (dd, *J* = 12.7 and 1.7 Hz, 1 H), 4.22 (dd, *J* = 12.7 and 4.1 Hz, 1 H), 7.10 (d, *J* = 8.4 Hz, 1 H), 7.50 - 7.53 (m, 2 H), 9.85 (s, 1 H); ^{1}H NMR (600 MHz, [D_6]acetone): δ = 1.32 (s, 3 H), 1.42 (s, 3 H), 3.86 (ddd, *J* = 7.3, 7.2 and 2.8 Hz, 1 H), 4.01 (dd, *J* = 12.3 and 7.3 Hz, 1 H), 4.27 (dd, *J* = 12.3 and 2.8 Hz, 1 H), 4.46 (d, *J* = 7.2 Hz, 1 H, OH), 7.11 (d, *J* = 8.2 Hz, 1 H), 7.45 (d, *J* = 1.8 Hz, 1 H), 7.54 (dd, *J* = 8.2 and 1.8 Hz, 1 H), 9.88 (s, 1 H). - ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.51, 25.21, 71.12, 75.12, 82.02, 122.62, 124.87. 16.52, 133.09, 152.42, 153.72, 190.74; ^{13}C NMR (150 MHz, [D_6]acetone): 21.23, 25.90, 71.50, 74.07, 81.99, 121.25, 124.36, 125.22, 132.89, 151.80, 153.12, 190.09. - MS (EI): *m/z* (%) = 222 (100) [M^+], 178 (20), 164 (30), 163 (69) [$C_9H_7O_3^+$], 152 (16), 149 (34), 138 (70) [$C_7H_6O_3^+$], 137 (49), 85 (14), 71 (27) [$C_4H_7O^+$], 59 (10) [$C_3H_7O^+$], 43.1 (25) [$C_3H_7^+$], 43.0 (13) [$C_2H_3O^+$], 41 (11). - Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.48; H, 6.55.

Preparation of the MTPA ester (*S,S*)-10. A suspension of (*S*)-**7** (56 mg, 0.25 mmol, 78% ee), (*R*)-MTPA chloride (63 mg, 0.25 mmol), $Sc(OTf)_3$ (74 mg, 0.15 mmol), and DMAP (36 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) was kept for 30 min at -8 °C. Then, pyridine (0.03 mL, 0.38 mmol) was added and the reaction mixture stirred for 30 min at -8 °C and then for 2 h at room temperature. The reaction mixture was filtered and the filtrate washed with 2 N HCl, aqueous $NaHCO_3$ and water (each 2 ×). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified by chromatography on a silica gel column (petroleum ether/EtOAc, 3:1) to give 27 mg (25%) of (*S,S*)-**10**. Colourless oil. - TLC: R_f = 0.57 (petroleum ether/EtOAc, 3:1). - GC/MS: R_i = 2659. - ^{1}H NMR (300 MHz, $CDCl_3$): major diastereomer, δ = 1.30 (s, 3 H), 1.44 (s, 3 H), 3.50 (m, 3H), 4.27 (dd, *J* = 12.9 and 5.3 Hz, 1 H), 4.53 (dd, *J* = 12.9 and 2.8 Hz, 1 H), 5.24 (dd, *J* = 5.3 and 2.8 Hz, 1 H), 6.96 (d, *J* = 8.2 Hz, 1 H), 7.38 (m, 5 H), 7.45 (d, 2.1 Hz, 1 H), 7.49 (dd, *J* = 8.2 and 2.1 Hz, 1 H), 9.84 (s, 1 H). - MS (EI): *m/z* (%) = 438.1304 (438.1290 calcd. for $C_{22}H_{21}F_3O_6$, 100) [M^+], 438 (29), 205 (30), 204 (39), 189 (100), 163 (16), 149 (25), 119 (8), 105 (11).

Preparation of the MTPA ester (*S,R*)-10. From (*R*)-**7** (77 mg, 0.35 mmol, 81% ee), (*R*)-MTPA-chloride (88 mg, 0.35 mmol), $Sc(OTf)_3$ (98 mg, 0.2 mmol), and DMAP (48 mg, 0.4 mmol) as described for (*S,S*)-**10**. Yield 46 mg (30%) of (*S,R*)-**10**. GC/MS: R_i = 2652. - ^{1}H NMR (300 MHz, $CDCl_3$): major diastereoisomer, δ = 1.20 (s, 3 H), 1.38 (s, 3 H), 3.59 (m, 3H), 4.38 (dd, *J* = 13.2 and 4.9 Hz, 1 H), 4.57 (dd, *J* = 13.2 and 2.5 Hz, 1 H), 5.22 (dd, *J* = 4.9 and 2.5 Hz, 1 H), 7.03 (d, *J* = 8.2 Hz, 1 H), 7.39 (m, 5 H), 7.45 (d, 2.0 Hz, 1 H), 7.51 (dd, *J* = 8.2 and 2.0 Hz, 1 H), 9.85 (s, 1 H). - MS (EI): *m/z* (%) = 438.1304 (438.1290 calcd. for $C_{22}H_{21}F_3O_6$, 100) [M^+], 438 (29), 205 (30), 204 (39), 189 (100), 163 (16), 149 (25), 119 (8), 105 (11).

Conversion of the strobilurins G, K and 9-methoxystrobilurin K to strobilurin I (1) and ozonolysis of 1 to aldehyde (*S*)-3. To a solution of the corresponding strobilurin (2.5 mg) in $CHCl_3$ (1 mL) 99% trifluoroacetic acid (TFA) (0.1 mL) was added. After stirring overnight at 20 °C, the solvent was evaporated under reduced pressure. The residue was purified by preparative TLC on SiO_2 with cyclohexane/EtOAc (1:1) and the resulting strobilurin I (**1**) identified by its optical rotation {$[\alpha]_D^{24}$ = +22.6 (*c* = 0.03, MeOH)} and ^{1}H NMR and MS data.

For ozonolysis, the crude residue from the TFA treatment of the corresponding strobilurin was dissolved in CH_2Cl_2 (10 mL), cooled to -78 °C and saturated with ozone. After 30 min, dimethyl sulfide (1 mL) was added and the solution allowed to warm to room temperature. The mixture was stirred for 2 h, the solvent evaporated and the residue purified by solid phase extraction on RP 18 with acetonitrile as eluent. After silylation with

N-methyl-*N*-(trimethylsilyl)trifluoroacetamide, the product was subjected to GC/MS analysis. The degradation product showed R_i = 1910, the TMS derivative of synthetic **3** R_i = 1910, and the TMS ether of aldehyde **14** R_i = 1915. The identity of the degradation products with aldehyde **3** was proven by co-injection.

GC/MS comparison of esters (*S,S*)-10 and (*S,R*)-10 with the MTPA ester of aldehyde 3 derived from the strobilurins. The degradation product **3** (1 mg) was dissolved in dry pyridine (30 μL). After addition of a catalytic amount of DMAP and (*R*)-MTPA-chloride (2 μL), the reaction mixture was kept for 5 h at 20 °C. A sample of the resulting mixture (0.5 μL) was used for GC/MS comparison. Synthetic (*S,S*)-**10**: R_i = 2659, synthetic (*S,R*)-**10**: R_i = 2652, (*S*)-MTPA ester derived from the natural products: R_i = 2659. The (*S*)-MTPA esters obtained from strobilurin D, I, K, and 9-methoxystrobilurin K were identical with synthetic (*S,S*)-**10**. This was confirmed in each case by co-injection.

Fermentation of *Mycena tintinnabulum* and isolation of strobilurin K (19). Fermentation was performed in 5 L Erlenmeyer flasks with YMG medium (2.5 L) at 22 °C and agitation (120 rpm). The flasks were inoculated with 20 mycelial pieces of a well-grown agar plate of the same medium. The antifungal activity of the mycelia was tested in the agar plate diffusion assay with *Mucor miehei* as test organism. After 41 days of fermentation, the mycelia (90 g) were separated from the culture fluid by filtration and extracted with 1:1 acetone/MeOH (2 L). After evaporation of the solvents, an oily crude extract (0.8 g) was obtained. This extract was applied onto a silica gel column (Merck 60, 0.063–0.2 mm, column 70 × 25 mm), and an enriched product (0.2 g) was obtained by elution with cyclohexane/EtOAc (8:2). Final purification of strobilurin K was achieved by preparative HPLC on Merck LiChrosorb DIOL (7 μm, column 250 × 25 mm) using cyclohexane/*t*-BuOMe (9:1) as eluent. Yield: 25 mg.

Strobilurin K (19): Oil. - $[\alpha]_D^{25}$: +1.6 (*c* = 0.22, MeOH). - CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 228 nm (0), 250 (-0.58), 281 (-0.92), 325 (0), 353 (+ 0.20). - UV (MeOH): λ_{max} = 206 nm (3.34), 279 (3.36), 292 nm (3.32, sh). ^{1}H and ^{13}C NMR see Table 2. - MS: *m/z* (%) = 443, 442.2354 (442.3355 calcd. for $C_{26}H_{34}O_6$, 100) [M^+], 305, 153 (10), 69 (10).

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