ASPERFURAN, A NOVEL ANTIFUNGAL METABOLITE FROM *ASPERGILLUS ORYZAE*†

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(Received for publication November 8, 1989)

Asperfuran is a novel antifungal dihydrobenzofuran derivative produced by a strain of *Aspergillus oryzae*. Asperfuran weakly inhibited chitin synthase from *Coprinus cinereus*. This inhibition could be abolished by the addition of egg lecithin. In the agar diffusion assay asperfuran induced morphological changes in *Mucor miehei* at very low concentrations (20 ng/disc) while growth was only partly inhibited. In HeLa S3 and L1210 cells it showed weak cytotoxicity, the IC_{50} was 25 μg/ml.

Screenings for chitin synthase inhibitors in the culture broth of actinomycetes have been performed by many groups[1~3]. But so far no inhibitor is reported from fungal cultures. Therefore a screening using the plate diffusion assay in combination with an enzymatic assay for chitin synthase was carried out. Extracts from 200 basidiomycetes, ascomycetes and imperfect fungi were tested. Strain HA 302-84 exhibiting strong antifungal activity and weak enzyme inhibition was identified as *Aspergillus oryzae*. A novel compound, named asperfuran, was isolated from the culture broth of this fungus.

In this paper the taxonomy of the producing strain, the production, isolation, structural elucidation and biological properties of asperfuran are presented.

Results and Discussion

Taxonomy of the Producing Strain

Strain HA 302-84 was isolated from a soil sample collected in China. Due to the yellow green color of the culture on Czapek-Dox and malt agar and according to the morphology of the conidiophores the strain was assigned to the genus *Aspergillus* and to the *Aspergillus flavus* group[4~6]. Comparison of the morphological characteristics reported for *Aspergillus parasiticus*, *A. flavus* and *A. oryzae* as given in Table 1 showed that our strain is close to *A. oryzae*. When cultures of strain HA 302-84 were grown on autoclaved rice or corn kernels, no production of fluorescent compounds, such as aflatoxins, could be detected.

Production of Asperfuran

For the production of asperfuran *A. oryzae*, HA 302-84, was grown on YMG-medium[7] at 23°C. A 12-liter fermenter containing 10 liters of medium was inoculated with conidia from one agar slant (9 cm i.d.) and cultivated for 48 hours with an agitation of 150 rpm and an aeration of 0.1 v/v/m. It was used as a seed culture for a 140-liter fermenter containing 90 liters of YMG-medium. The conditions were the

† This paper is dedicated to Prof. Dr. H. ZÄHNER on the occasion of his 60th birthday.

Table 1. Morphological features of *Aspergillus oryzae*, HA 302-84: Comparison with other strains of the *Aspergillus flavus* group.

Species	Conidia		Conidiophores length (μm)	Vesicle i.d. (μm)	Mono-	Bise-
	Surface	i.d. (μm)			riate	
Aspergillus parasiticus[5]	Echinulate	(3.5) 4.0~7.0	(170) 250~ 470 (920)	15~32	++	+
A. flavus[5]	Smooth-rough	3.0~7.0 (8.0)	340~1,650	22~48	+	++
A. oryzae[5]	Smooth	3.5~8.5	680~5,900	24~53	+	++
A. oryzae HA 302-84	Smooth	(4.5) 6.0~8.0	(180) ~4,000	(25) 40~50 (70)	+	++
A. oryzae[6]	Smooth-rough	4.5~8.0	~5,000	40~80	+	+
A. flavus[6]	Echinulate	3.6	~2,500	25~45	+	+
A. parasiticus[6]	Echinulate	3.5~5.5	300~700	20~30	+	−

same as for the seed culture. The production of the metabolite was monitored by the agar diffusion assay using *Mucor miehei* as the test organism. Maximum metabolite production was reached after 40~50 hours culture, when the carbon sources were used up. The antifungal activity was located in the culture filtrate. The mycelia were discarded.

The culture filtrate (85 liters) was extracted with EtOAc (2×15 liters). After evaporation of the solvent the remaining extract (5.1 g) was adsorbed on silica gel and loaded onto a column with silica gel (66×200 mm) in cyclohexane - EtOAc (80 : 20). After washing with 2 liters of the same solvent, the antibiotic was eluted with 2 liters of cyclohexane - EtOAc (70 : 30). Recrystallization from the same solvent afforded asperfuran as white needles (185 mg).

Table 2. Selected ^{13}C NMR data di-*O*-methylasperfuran (**2**) and the model compounds **5** and **6** (100.6 MHz in $CDCl_3$, δ, ppm).

C-Atom	**2**	**5**	**6**
C-2	84.14	84.02	84.49
C-3	37.20	36.61	32.83
C-3a	127.67	127.30	105.27
C-4	101.11	100.99[a]	161.58[b]
C-5	154.70	154.69	88.31[c]
C-6	99.11	99.01[a]	161.22[b]
C-7	144.42	144.31	91.04[c]
C-7a	141.83	141.67	156.56
OCH_3	55.90	55.81	55.26
OCH_3	55.98	55.81	55.49

[a~c] Assignments may be reversed.

Asperfuran is easily soluble in MeOH and acetone and fairly soluble in EtOAc.

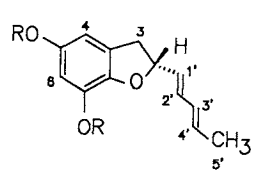
Structural Elucidation

Asperfuran, $C_{13}H_{14}O_3$, forms a dimethyl ether **2** and a diacetate **3** which indicates the presence of two phenolic hydroxy groups. From the 1D-^{1}H NMR data and COSY-90 experiments the proton sequence for a $H_3C-CH=CH-CH=CH-CH(OR)-CH_2-$ unit with two *trans* double bonds can be deduced. The ^{13}C NMR spectrum shows additional signals for a tetrasubstituted benzene ring which leads to two possible dihydrobenzofuran structures **1** and **4** for asperfuran.

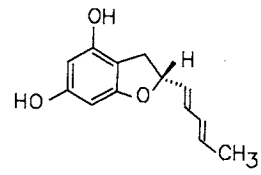
A distinction between these formulas was made by comparison of the ^{13}C NMR data of dimethyl ether **2** with those of the synthetic dihydrobenzofurans **5** and **6** (Table 2). The model compounds were prepared from alkali salts of 1-hydroxy-2,4-dimethoxybenzene and 1-hydroxy-3,5-dimethoxybenzene, respectively, by treatment with (*E*)-1,4-dibromo-2-butene in toluene[8].

The ^{13}C NMR data of di-*O*-methylasperfuran (**2**) show excellent agreement with those of 5,7-dimethoxy-2-vinyldihydrobezofuran (**5**) which proves formula **1** for the antibiotic.

Recently, an enantioselective cyclization of 2-(2-butenyl)phenols with a chiral palladium reagent in the presence of $Cu(OAc)_2$ has been developed by HOSOKAWA *et al.*[9] This method, albeit of low enantioselectivity, allows the synthesis of 2-vinyl-dihydrobenzofurans of predictable absolute configuration.



1 R=H
2 R=CH_3
3 R=Ac



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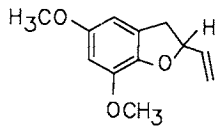
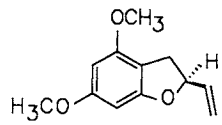
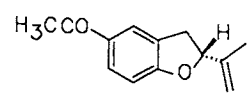


5



(*S*)-6



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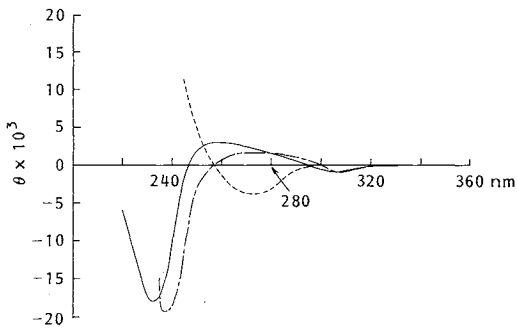
In the case of (*E*)-2-(2-butenyl)-3,5-dimethoxyphenol the cyclization leads to (*S*)-(+)-dihydrobenzofuran **6**, whereas experiments to prepare (*S*)-**5** by the same method were unsuccessful. A comparison of the CD spectra (Fig. 1) revealed that the CD curve of (*S*)-**6** is opposite to that of asperfuran (**1**) and its dimethyl ether **2** which allows the assignment of the (*R*)-configuration to the natural product. This is in accord with the fact that asperfuran (**1**) and (*R*)-tremetone[10] (**7**) both exhibit a negative optical rotation. The absolute configuration of the latter compound has been determined by degradation to (*S*)-malic acid[10].

Fig. 1. CD spectra of asperfuran (**1**), di-*O*-methyl-asperfuran (**2**) and (*S*)-4,6-dimethoxy-2-vinyldihydrobenzofuran (**6**).

1 ——, **2** —·—, **6** ------.



Biological Properties

Asperfuran (**1**) at a concentration of 300 μM reduced the chitin synthase activity to 50%. This inhibition was completely abolished by the addition of egg lecithin (8 mg/ml). Glucan synthase prepared from mycelia of *Schizophyllum commune* was not inhibited.

Bacteria like *Bacillus brevis*, *Bacillus subtilis*, *Micrococcus luteus*, *Acinetobacter calcoaceticus*, *Staphylococcus aureus* and *Escherichia coli* were not affected by 50 μg/ml of asperfuran.

The antifungal spectrum of **1** in the plate diffusion assay is given in Table 3. In all cases the inhibition zones were incomplete and there was little difference whether spores or hyphal fragments were assayed. Mycelia of *M. miehei* showed morphological changes in the presence of asperfuran, starting at 20 ng/disc. Fig. 2 shows the typical picture obtained with 1 μg/disc. Mycelia grown in submerged culture were less sensitive. At 20 μg/ml asperfuran reduced growth of *M. miehei* to 50%. At this concentration no effect on the incorporation of adenine into DNA (alkali resistant fraction), leucine, uridine and acetate into acid-insoluble fraction mycelia was observed. Incorporation of glucose and GlcNAc was reduced to 70% compared to the control without antibiotic.

At 25 μg/ml asperfuran inhibited the proliferation of HeLa S3 and L1210 cells to 50%. Ehrlich ascites tumor cells were not affected by 50 μg/ml of the compound.

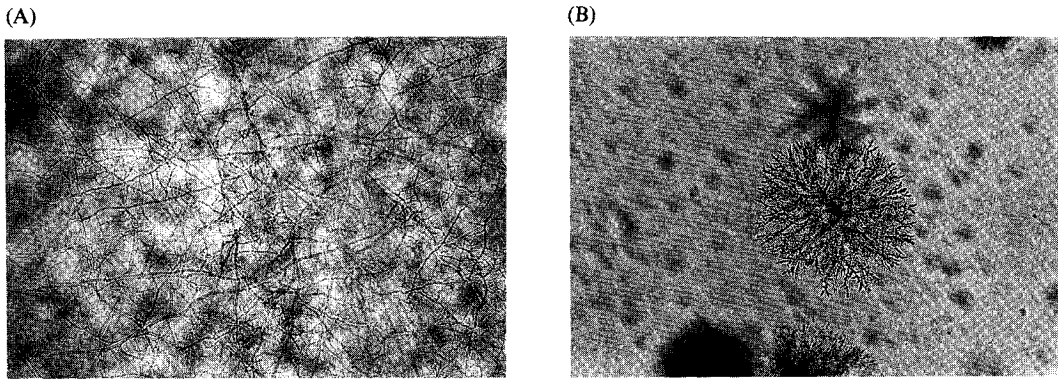
Table 3. Antifungal activity of asperfuran in the agar diffusion assay.

Organism	Inhibition zone[a] (mm)		
	50 μg/disc	10 μg/disc	1 μg/disc
Absidia glauca (+)	32	29	—
A. glauca (−)	28	20	—
A. glauca (−) conidia	37	29	17
Alternaria porri	30	23	—
Botrytis cinerea	20	—	
Candida albicans	—		
Cladosporium cladosporioides	13	—	
Curvularia lunata	17	7	—
Epicoccum purpurascens	18	7	—
Fusarium fujikuroi	7	—	
F. oxysporum	—		
Mucor hiemalis	16	—	
M. hiemalis conidia	13	—	—
M. miehei	34	34	28
M. miehei conidia	54	54	38
Nematospora coryli	9	—	
Neurospora crassa	7	—	
Paecilomyces varioti	24	18	—
P. varioti conidia	26	15	—
Phytophthora infestans	27	12	—
Pythium debaryanum	21	14	—
Rhodotorula glutinis	7	—	
Saccharomyces cerevisiae IS1	—		
Thamnidium anomalum	27	18	—
T. anomalum conidia	32	25	7
Ustilago nuda	—		
Venturia cerasi	18	7	—

[a] All inhibition zones were not clear.

Young mycelia grown for 14 hours were suspended in YMG - agar. Cell density: 5×10^5 (conidia or germinated conidia).

Fig. 2. Pictures of (A) Mycelia of *Mucor miehei* on YMG-agar (control) and (B) mycelia of *M. miehei* grown in the presence of 1 μg/ml asperfuran.



In *M. miehei* the oxygen uptake was not affected by asperfuran. Mitochondria isolated from bovine liver were not uncoupled by asperfuran and no hemolytic activity could be detected. Methylation leads to the dimethyl ether **2** completely devoid of antifungal activity. The inhibitory effect on chitin synthase

remained the same as for the mother compound indicating that the antifugal activity of asperfuran is not related to the inhibition of chitin synthase.

Dihydrofurans with weak antifungal and weak algicidal activities have been reported from the cultures of *Heterobasidion annosum*[11,12]. *Stereum subpileatum*, another basidiomycete, is known to produce benzofurans[13]. These metabolites are considered as being derived form the shikimic acid and mevalonate pathways, whether asperfuran has the same biogenetic origin remains to be established. Its substitution pattern however favors the polyketide route.

Experimental

Biological Assays

Preparation of partially purified chitin synthase from the mycelia of *Coprinus cinereus* and the enzyme assay were performed as described previously[14]. The specific activity of the enzyme used was 260 nmol/mg·minute. For antimicrobial assays, the bacteria were grown in nutrient broth (Difco), the fungi in YMG-medium[7], the same medium was used for fungal cultures in screening. HeLa S3 cells (ATCC CCL 2.2) were grown as described by MIRABELLI *et al.*[15], L1210 cells (ATCC CCL 219) as described before[16]. The effects of asperfuran on macromolecular syntheses were tested in a similar way as described by ANKE *et al.*[17]. Incorporation of precursors into TCA-precipitable material in freshly germinated conidia of *M. miehei* was followed as described by HAMAMATO *et al.*[18]. Mitochondria were prepared from bovine liver according to CLARK[19]. Oxygen-uptake was measured polarographically with a Clark electrode. Hemolytic activity was tested according to KINSKY[20].

General

The mp's were determined with a Reichert hot-plate microscope and are uncorrected. Spectral data were recorded on the following instruments: NMR, Bruker AM-400; IR, Perkin-Elmer 1420; UV, Varian Cary-17; CD, Jouan-Roussel III; MS, A.E.I. MS-50 and MS-30 at 70 eV. TLC was carried out on aluminium foils, coated with Silica gel 60 F_{254} Merck, Darmstadt, No. 5554.

Asperfuran (1)

Colorless crystals, mp 130°C; $[\alpha]_D^{20}$ −20.9° (*c* 0.21, acetone); Rf 0.61 (toluene-acetone-AcOH, 70:30:1), 0.25 (cyclohexane-2-propanol, 9:1); UV λ_{max}^{MeOH} nm (log ε) 227 (4.51), 295 (3.57); CD $\lambda_{extreme}^{MeOH}$ nm (θ) 232 (-18.43×10^3), 247 (0), 260 ($+3.02\times10^3$), 294 (0), 305 (-0.86×10^3); IR (KBr) cm^{-1} 3625 (m), 3600~3100 (sst), 2910 (m), 2845 (w), 1660 (m), 1635 (s), 1610 (s), 1500 (s), 1470 (sst), 1440 (sh), 1380 (w), 1350 (m), 1315 (s), 1270 (m), 1260~1210 (br), 1190 (w), 1160 (s), 1130 (sst), 1080 (w), 1050 (s), 1010 (w), 990 (s), 970 (m), 940 (m), 850 (s), 840 (sh), 810 (s), 710 (w), 695 (w), 650 (m); EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 219 (17), 218.0946 (100, M^+) calcd for $C_{13}H_{14}O_3$ 218.0943, 203 (24, $C_{12}H_{12}O_3$), 189 (9), 185 (12), 177 (8), 164 (12), 163 (61, $C_9H_7O_3$), 157 (6), 139 (32, $C_7H_7O_3$), 138 (60, $C_7H_6O_3$), 128 (7), 115 (7), 111 (7), 110 (17, $C_6H_6O_2$), 91 (12), 81 (8), 80 (9), 79 (15), 77 (13), 65 (6); ^1H NMR (400 MHz, acetone-d_6) δ 1.72 (d, J=7 Hz, 5'-H), 2.85 (br dd, J=15 and 8 Hz, 3-H_b), 3.23 (br dd, J=15 and 8.5 Hz, 3-H_a), 5.08 (br ddd, J=8.5, 8 and 7.5 Hz, 2-H), 5.71 (dd, J=15 and 7.5 Hz, 1'-H), 5.76 (m, 4'-H), 6.09 (br dd, J=15 and 11 Hz, 3'-H), 6.17, 6.20 (each m, 4-H, 6-H), 6.28 (dd, J=15 and 11 Hz, 2'-H), 7.78, 7.87 (each br s, 2-OH). The assignments given have been proven by ^1H, ^1H-COSY experiments. ^{13}C NMR (100.6 MHz, acetone-d_6) δ 18.15 (qdd, J=126, 6 and 4 Hz, C-5'), 37.81 (tm, J=134 Hz, C-3), 83.89 (dm, J=150 Hz, C-2), 103.32 (br d, J=157 Hz, C-4 and C-6), 128.90 (td, J=6 and 1.5 Hz, C-3a), 130.86 (dm, J=156 Hz, C-1'), 131.06 (dqd, J=151, 7 and 4 Hz, C-4'), 131.64 (ddq, J=154, 7 and 7 Hz, C-3'), 132.79 (dm, J=155 Hz, C-2'), 140.70 (br m, C-7a), 141.96 (m, C-7), 152.76 (m, C-5). The assignments given have been proven by 2D ^1H-^{13}C chemical shift correlation.

Di-*O*-methylasperfuran (2)

To a solution of asperfuran (**1**) (12 mg) in MeOH (5 ml) etheric diazomethane (*ca.* 1 ml) was added and the mixture stirred for 3 hours at 20°C. The mixture evaporated and the residue separated form small

quantities of the monomethyl ether by chromatography on a SepPak-SiO_2-cartridge (eluant CH_2Cl_2). **2** (10.8 mg) was obtained as colorless crystals: MP 104°C; $[\alpha]_D^{20} -33.0°$ (*c* 0.1, CH_2Cl_2); Rf 0.75 (CH_2Cl_2); UV λ_{max}^{dioxan} nm (log ε) 230 (4.39), 295 (3.46); CD $\lambda_{extreme}^{dioxan}$ nm (θ) 237 (-19.47×10^3), 256 (0), 274 ($+1.83 \times 10^3$) 300 (0), 307.5 (-1.03×10^3); IR (KBr) cm^{-1} 3020 (sh), 3000 (w), 2960 (m), 2930 (s), 2855 (w), 2830 (sh), 1660 (w), 1635 (sh), 1615 (sh), 1600 (s), 1495 (sst), 1470 (m), 1460 (m), 1450 (m), 1440 (m), 1385 (w), 1340 (m), 1315 (m), 1260 (m), 1215 (sh), 1210 (sst), 1140 (s), 1100 (sst), 1045 (s), 995 (s), 940 (m), 860 (m), 835 (m), 815 (s), 805 (sh), 720 (w); EI-MS (direct inlet, 180°C): *m/z* (relative intensity %) 247 (18), 246.1257 (100, M^+) calcd for $C_{15}H_{18}O_3$ 246.1257, 231 (20), 199 (14), 192 (16), 191 (39), 167 (33), 166 (34), 71 (12), 69 (11), 57 (19), 55 (11), 43 (10); ^1H NMR (400 MHz, $CDCl_3$) δ 1.75 (dd, $J=6.7$ and 1.5 Hz, 5'-H), 2.97 (br dd, $J=15$ and 8.5 Hz, 3-H_b), 3.28 (br dd, $J=15$ and 9 Hz, 3-H_a), 3.73, 3.81 (each s, 2-OCH_3), 5.19 (br "q", $J \approx 8.5$ Hz, 2H), 5.71 (dd, $J=15$ and 7 Hz, 1'-H), 5.74 (m, 4'-H), 6.03 (dd, $J=15$ and 10.5 Hz, 3'-H), 6.25 (dd, $J=15$ and 10.5 Hz, 2'-H), 6.33 ("s", 4-H and 6-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.16 (qdd, $J=126$, 7 and 5 Hz C-5'), 37.20 (t, $J=135$ Hz, C-3) 55.90 (q, $J=148$ Hz, OCH_3), 55.98 (q, $J=146$ Hz, OCH_3), 84.14 (br d, $J=151$ Hz, C-2), 99.11 (dd, $J=157$ and 5 Hz, C-6), 101.11 (ddm, $J=162$ and 5 Hz, C-4), 127.67 (t, $J=6$ Hz, C-3a), 128.92 (br d, $J=149$ Hz, C-1'), 130.49 (dm, $J=150$ Hz, C-3' or C-4'), 131.14 (dm, $J=150$ Hz, C-3' or C-4'), 132.98 (dm, $J=151$ Hz, C-2'), 141.83 (m, C-7a), 144.42 (m, C-7), 154.70 (br d, $J=4$ Hz, C-5).

Di-*O*-acetylasperfuran (**3**)

Asperfuran (**1**) (5 mg) and a catalytic amount of 4-(demethylamino)pyridine (DMAP) were dissolved in a mixture of acetic anhydride (5 ml) and pyridine (0.1 ml) and stirred for 12 hours at 20°C. After hydrolysis of the mixture with ice, the solution was concd *in vacuo* and chromatographed on silica gel with acetone-CCl_4 (1 : 1). **3** (2.2 mg) was obtained as a yellowish oil which still contained traces of impurities: Rf 0.91 (acetone-CCl_4, 1 : 1); UV λ_{max}^{MeOH} nm 235, 287; EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 302.1131 (9, M^+) calcd for $C_{17}H_{18}O_5$ 302.1154, 260 (30), 219 (100), 203 (18), 163 (38), 150 (10), 139 (21), 138 (54), 43 (35); ^1H NMR (400 MHz, $CDCl_3$) δ 1.75 (dd, $J=6.5$ and 1.5 Hz, 5'-H), 2.22, 2.26 (each s, 2-$COCH_3$), 3.00 (ddt, $J=16$, 8 and 1 Hz, 3-H_b), 3.37 (ddt, $J=16$, 9 and 1 Hz, 3-H_a), 5.25 (br "q", $J \approx 8.5$ Hz, 2-H), 5.68 (ddq, $J=15$, 7.75 and 1.3 Hz, 1'-H), 5.77 (dq, $J=15$ and 6.5 Hz, 4'-H), 6.03 (ddq, $J=15$, 10.5 and 1.5 Hz, 3'-H), 6.26 (dd, $J=15$ and 10.5 Hz, 2'-H), 6.65, 6.76 (each dm, $J=2.5$ Hz, 6-H and 4-H).

(±)-5,7-Dimethoxy-2-vinyldihydrobenzofuran (**5**)

A solution of 2,4-dimethoxyphenol (1.0 g) in toluene (40 ml) and sodium (0.15 g) was refluxed for 3 hours under argon. (*E*)-1,4-Dibromo-2-butene (1.4 g) was added and the resulting slurry was heated under reflux for 18 hours. After cooling to 20°C, the reaction mixture was quenched with an excess of aq NH_4Cl, extracted with Et_2O (3×50 ml) and dried over Na_2SO_4. The solvent was evaporated, and the residue obtained was chromatographed on silica gel eluting with petroleum ether-EtOAc (20 : 1) to give **5** (215 mg, 19%) as a colorless oil: HR-MS (direct inlet, 180°C) *m/z* 206.0934 (M^+), calcd for $C_{12}H_{14}O_3$ 206.0943; ^1H NMR (400 MHz, $CDCl_3$) δ 2.80 (1H, dddd, $J=15.3$, 8.3, 0.8 and 0.8 Hz), 3.30 (1H, dddd, $J=15.3$, 9.3, 1 and 1 Hz), 3.73 (s, OCH_3), 3.82 (s, OCH_3), 5.19 (1H, ddddd, $J=9.3$, 8.3, 7.3, 2.5 and 2.5 Hz), 5.20 (1H, ddd, $J=10.5$, 2.5 and 2.5 Hz), 5.35 (1H, ddd, $J=17$, 2.5 and 2.5 Hz), 6.03 (1H, ddd, $J=17$, 10.5 and 7 Hz), 6.33 (2H, m).

4,6-Dimethoxy-2-vinyldihydrobenzofuran (**6**)

Racemate: A solution of 3,5-dimethoxyphenol (1.54 g) was treated with *n*-butyllithum (6.25 ml; 1.6 M hexane solution) under argon at 20°C. After stirring for 30 minutes, (*E*)-1,4-dibromo-2-butene (2.14 g) in toluene (10 ml) was added and the reaction mixture was refluxed for 22 hours. The work-up as described for **5** yielded **6** (70 mg, 21%) as a colorless oil: HR-MS (direct inlet, 180°C) *m/z* 206.0935 (M^+), calcd for $C_{12}H_{14}O_3$ 206.0943; ^1H NMR (400 MHz, $CDCl_3$) δ 2.87 (1H, dd, $J=15$ and 7.5 Hz), 3.28 (1H, dd, $J=15$ and 9.5 Hz), 3.73 (s, OCH_3), 3.75 (s, OCH_3), 5.20 (1H, ddd, $J=9.5$, 7.5 and 7.5 Hz), 5.20 (1H, d, $J=9.5$ Hz), 5.34 (1H, d, $J=17$ Hz), 5.95 (1H, ddd, $J=17$, 9.5 and 7.5 Hz), 5.98 (1H, d, $J=2$ Hz), 6.05 (1H, d, $J=2$ Hz).

(*S*)-Enantiomer[9]: (+)-Bis (acetoxy(3,2,10-η-pinene)palladium(II)) (74.2 mg) and $Cu(OAc)_2$ (45 mg; dried at 85°C/2 mmHg) were warmed to 35°C. After flashing the flask with oxygen, a solution of (*E*)-2-(2-butenyl)-3,5-dimethoxyphenol (520 mg) in MeOH (10 ml) was added and the mixture was stirred

for 6 hours. The solvent was evaporated, the residue taken up in Et_2O and washed twice with water. The organic layer was dried (Na_2SO_4) and the solvent evaporated. Filtration over a short silica gel column (*n*-hexane) followed by Kugelrohr distillation and preparative TLC (SiO_2, toluene-$CHCl_3$, 1:1) yielded (*S*)-**6** (275 mg, 53.5%): $[\alpha]_D^{20} + 5.0°$ (*c* 0.6, $CHCl_3$); CD see Fig. 1. The optical purity of the product was not determined.

Acknowledgments

We thank A. HELFER, Kaiserslautern for expert technical assistance. This work was supported by grants from the Dechema, Frankfurt, from the BASF AG, Ludwigshafen, and from the Bundesministerium für Forschung und Technologie.

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