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Supporting Information

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Biocatalytic Single-step Alkene Cleavage of Aryl Alkenes, an Enzymatic Equivalent to Reductive Ozonization

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Contents

General

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^1H) and 90 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00), coupling constants (*J*) are given in Hz.

TLC plates were run on silica gel Merck 60 (F254) and compounds were visualized by standard techniques. Aldehydes were visualized using 2,4-dinitrophenylhydrazine (0.4% w/v in 2N HCl).

Flash chromatography was performed on silica gel Merck 60 (230-400 mesh). Petroleum ether, acetone and ethyl acetate were used as eluent. Solvents were dried and freshly distilled by common practice. Petroleum ether (p.e.) had a boiling range of 60-90 °C unless otherwise noted.

GC analyses were carried out on a Varian 3900 gas chromatograph equipped with FID and a HP 1701 capillary column (30 m, 0.25 mm, 0.25 µm film, N_2).

GC/MS analyses were carried out on a Hewlett Packard 6890 equipped with FID and a HP Mass Selective Detector 5973. The GC was attached with a HP 5 MS capillary column (30 m, 0.25 mm, 0.25 µm film) and Helium was used as carrier gas, the column head pressure was 9.65 psi.

Cultivation of *Trametes hirsuta G FCC 047*

Complex medium: 15 g L^{-1} D-glucose-anhydrous, 3 g L^{-1} L-asparagin-monohydrate, 1.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4.7\ H_2O$, 6 g L^{-1} yeast extract, 0.1 mL L^{-1} 3,4-dimethoxybenzyl alcohol, 1 mL L^{-1} trace element solution [80 mg L^{-1} $FeCl_3.6H_2O$ (Fluka 44944), 90 mg L^{-1} $ZnSO_4.7H_2O$ (Aldrich 22,137-6), 30 mg L^{-1} $MnSO_4.H_2O$, 5 mg L^{-1} $CuSO_4.5H_2O$ (Aldrich 46,913-0), 0.4 g L^{-1} EDTA]. Pre-culture: the fungus was grown on the complex medium (100 mL) in non-baffled Erlenmeyer flasks (300 mL) at 25 °C at 150 rpm for 7 days. Fermentation culture: fresh medium (300 mL in 1 L shake flasks) was inoculated with 15 mL of the homogenized pre-culture (Heidolph, DIAX 900, 20 sec, power 3), and incubated at 25 °C at 150 rpm. After 5 days the cells were harvested by centrifugation (8000 rpm, 4 °C, 20 min), washed with buffer (Tris-HCl, 10 mM, pH 7.5), suspended in a minimum amount of buffer (Tris-HCl, 50 mM, pH 7.5), shock frozen with liquid nitrogen and lyophilized.

Assay

Lyophilized cells (25 mg) were rehydrated in Bis-Tris buffer (900 µL, 50 mM, pH 6) on a rotary shaker (25 °C, 150 rpm) for 30 min. Cells were removed by centrifugation (4 °C, 13000 rpm, 2 min) and the supernatant (900 µL, 1 mg protein mL^{-1}), was transferred into a glass vial (1.5 mL) (freeze drying was used to break the cell wall, since the enzyme is intracellular). Substrate (6 µL) was added and the vial was immediately closed with a metal cap after hydrogen peroxide (100 µL, 1 M) was added. The mixture was shaken on a rotary shaker (25 °C, 150 rpm). After 24 hours the mixture was extracted with ethyl acetate (2 x 500 µL) containing toluene as an internal standard (6 µL toluene mL^{-1} ethyl acetate) and centrifuged (4 °C, 13000 rpm, 2 min) for phase separation. The combined organic layers were dried over Na_2SO_4 and analyzed on GC and GC/MS.

Variation of the co-solvent concentration: As above using *t*-anethole (6 µL, 3.9 µmol) as substrate, taking an appropriate amount of the supernatant (1 mg protein mL^{-1}) and an appropriate amount of ethanol.

Variation of the substrate concentration: In analogy to the procedure above but with 350 µL supernatant (1 mg protein mL^{-1}) and ethanol (150 µL). The amount of substrate and buffer to reach one milliliter of reaction mixture was chosen as appropriate.

Experiments with $^{18}O_2$

Indene **1b** is cleaved to its corresponding di-aldehyde **2b**, thus both cleavage sites are in the same product molecule. Figure S1 shows the MS-spectrum of the di-aldehyde by using $^{16}O_2$ as oxidant.

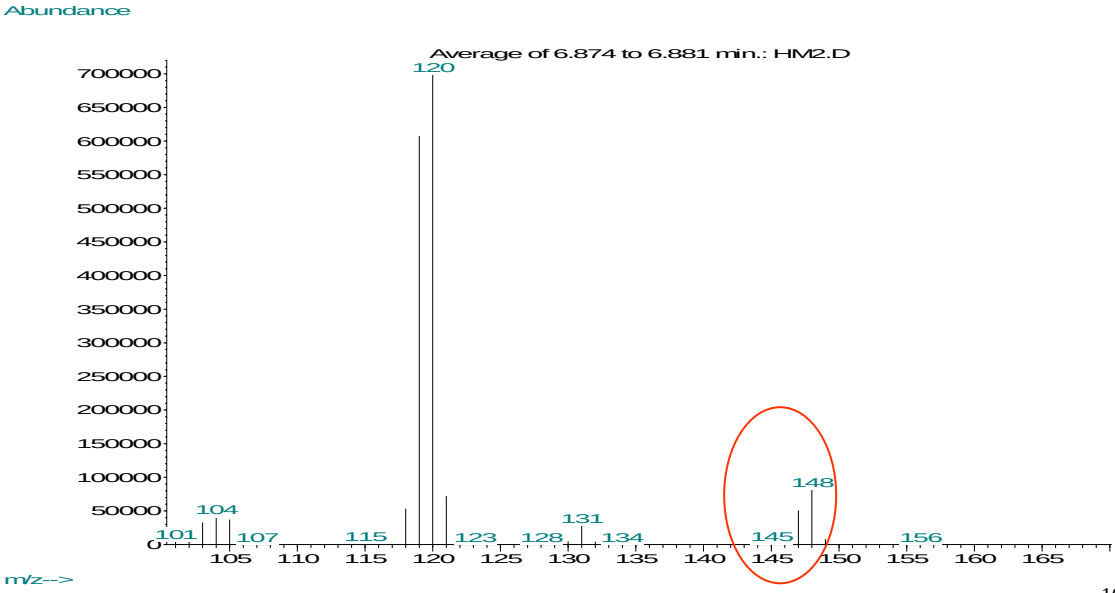


Figure S1. GC-MS data from the indene **1b** cleavage product (di-aldehyde **2b**) using $^{16}O_2$ and H_2O^{16} (MW 148 g mol^{-1}).

Labeling experiments using $^{18}O_2$ for the transformation of indene resulted in an increase of molecular mass of 4 g mol^{-1}, indicating the incorporation of 2 atoms ^{18}O from $^{18}O_2$ (Figure S2).

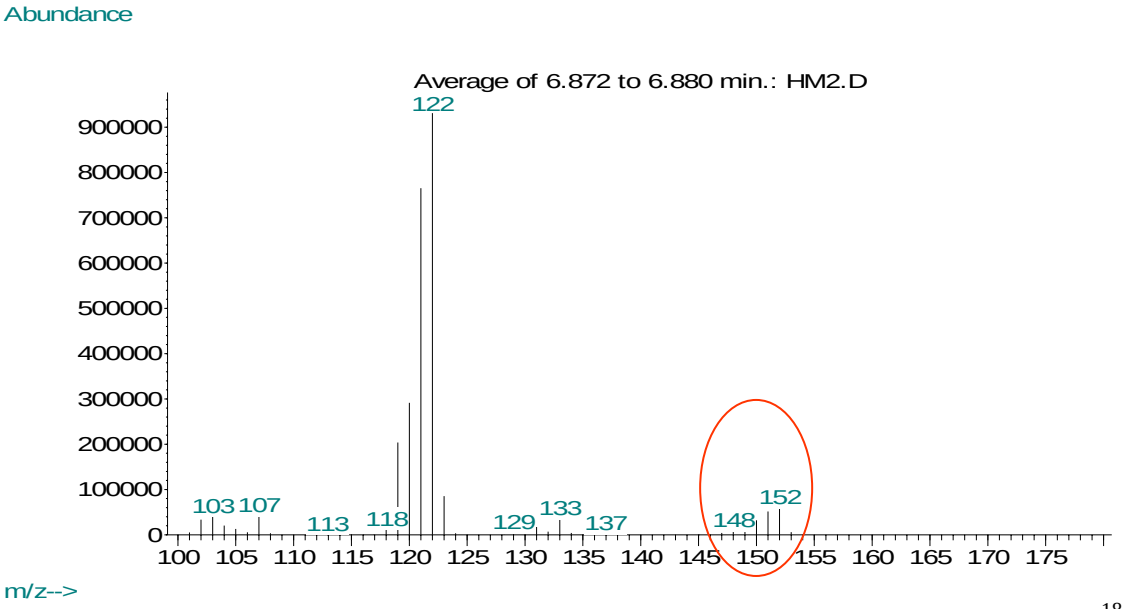


Figure S2. GC-MS data from the indene **1b** cleavage product (di-aldehyde **2b**) using $^{18}O_2$ and H_2O^{16} (MW 152 g mol^{-1}).

Table S1 lists the data of the two experiments of Figure S1 and S2, showing again the shift in m/z values of 4 when employing $^{18}O_2$, indicating the incorporation of 2 atoms ^{18}O from $^{18}O_2$.

	$^{16}O_2$		**$^{18}O_2$**	
m/z	**abundance**	**rel. intensity [%]**	**abundance**	**rel. intensity [%]**
145	728	1	-	-
146	5527	7	-	-
147	63034	75	3514	7
148	84078	100	7129	12
149	8647	10	24584	42
150	1021	1	31972	54
151	-	-	51588	88
152	-	-	58524	100
153	-	-	5812	9.9
154	-	-	274	0.4

Relative intensities only correspond to area of molecule peak

Table S1. Molecular weight distribution unlabeled/labeled 2-(2-oxo-ethyl)-benzaldehyde **2b** determined *via* GC-MS.

Control Experiment: Conversion of Indene to 2-(2-oxo-ethyl)-benzaldehyde in H_2O^{18} Using $^{16}O_2$

Experiments were performed similar as described above but using a buffer prepared with H_2O^{18}. Molecular oxygen $^{16}O_2$ was used as an oxidant.

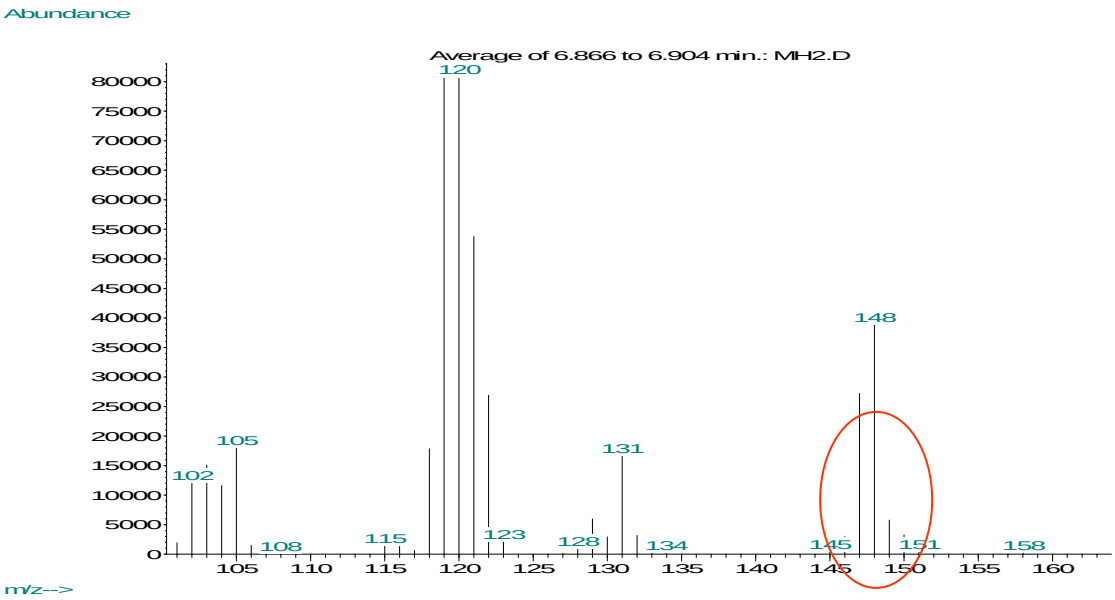


Figure S3. GC-MS data from the indene **1b** cleavage product (di-aldehyde **2b**) using $^{16}O_2$ and H_2O^{18} (MW 148 g mol^{-1}).

	$^{16}O_2/H_2O^{16}$		$^{16}O_2/H_2O^{18}$	
m/z	**abundance**	**rel. intensity [%]**	**abundance**	**rel. intensity [%]**
145	728	1	323	1
146	5527	7	2871	9
147	63034	75	21449	67
148	84078	100	31909	100
149	8647	10	4856	15
150	1021	1	2916	9

Relative intensities only correspond to area of molecule peak

Table S2. Molecular weight distribution of 2-(2-oxo-ethyl)-benzaldehyde **2b** determined via GC-MS produced in unlabeled/labeled buffer.

No ^{18}O incorporation was found using H_2O^{18} indicating that the two oxygen atoms originate from molecular oxygen and that it is a single step process.

General procedure for the biocatalytic alkene cleavage using $^{18}O_2$

Lyophilized cells (25 mg) of *Trametes hirsuta* G were rehydrated in Bis-Tris/HCl buffer (900 µL, 50 mM, pH 6) for 30 min on a shaker (150 rpm). Cells were removed by centrifugation (13000 rpm, 2 min) and supernatant (900 µL) was transferred into an Eppendorf vial (1.5 mL). Indene (6 µL) was transformed to the corresponding aldehyde using $^{18}O_2$ as the main oxidant. Once per hour oxygen (10 mL) was bubbled through the reaction mixture within one minute, in summary the reaction was aerated seven times during the first seven hours. The mixture was shaken on a rotary shaker (150 rpm, 25 °C). After 24 h the mixture was extracted with EtOAc (2 x 500 µL) containing toluene as an internal standard (6 µL toluene mL^{-1} EtOAc) and centrifuged (4 °C, 13000 rpm, 2 min) for phase separation. The combined organic layers were dried over Na_2SO_4 and analyzed by GC/MS. Blank experiments using air and experiments using $^{16}O_2$ were performed in comparison.

General procedure for the biocatalytic alkene cleavage using H_2O^{18}

Lyophilized cells (25 mg) of *Trametes hirsuta* G were rehydrated in Bis-Tris/HCl buffer (900 µL, 50 mM, pH 6) prepared with H_2O^{18} for 30 min on a rotary shaker (150 rpm). Cells were removed by centrifugation (13000 rpm, 2 min) and supernatant (900 µL) was transferred into an Eppendorf vial (1.5 mL). Substrate (6 µL) was transformed to the corresponding aldehyde using $^{16}O_2$ as the main oxidant. Once per hour oxygen (10 mL) was bubbled through the reaction mixture within one minute; in summary the reaction was aerated 7 times. The mixture was shaken on a rotary shaker (150 rpm, 25 °C). After 24 hours the mixture was extracted with EtOAc (2 x 500 µL) containing toluene as an internal standard (6 µL toluene mL^{-1} EtOAc) and centrifuged (13000 rpm, 2 min) for phase separation. The combined organic layers were dried with Na_2SO_4 and analyzed with GC/MS.

Synthesis of substrate and reference material

All substrates (except **1a**) and reference aldehydes (except **2b**) were purchased from Aldrich, Lancaster and Acros with highest purity available.

*(E)-1-phenyl-1-butene **1a***

Copper iodide (2.3 g, 0.012 mol) was added to freshly prepared methyl magnesium iodide (prepared from magnesium (7.0 g, 0.29 mol) and methyl iodide (41.2 g, 0.29 mol, 18.1 mL) in Et_2O (150 mL) at –20 °C and allowed to stir at room temperature for 1 hour.[1] The commercially available chloride (*E*)-3-chloro-1-phenyl-1-propene (31.3 g, 0.19 mol) in Et_2O (50 mL) was added slowly at –20 °C and stirred at room temperature for 18 hhours. The reaction mixture was slowly quenched with aqueous saturated NH_4Cl-solution (100 mL) and extracted with Et_2O (3 x 50 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. Solvent removal in vacuo furnished the crude product. Vacuo destillation (b.p.: 44 °C, 6 mbar) gave (*E*)-1-phenyl-1-butene **2a** (9.46 g, 37%) as colorless liquid. R_f (petroleum ether/EtOAc 10:1) = 0.65; ^{1}H-NMR: 1.15 (t, 3H, *J*=7.4 Hz), 2.28 (m, 2H), 6.32 (dt, 1H, J_1=15.8 Hz, J_2=6.1 Hz), 6.43 (d, 1H, *J*=15.8 Hz), 7.21-7.40 (m, 5H); ^{13}C-NMR: 13.6, 26.1, 125.9, 126.7, 128.5, 128.8, 132.6, 137.9

^{1}H-NMR identical to literature.[2]

*Preparation of di-aldehyde 2-(2-oxo-ethyl)-benzaldehyde **2b***

A solution of the indene oxide (0.2 mmol, 26.4 mg) and ceric ammonium nitrate (0.21 mmol) in CH_3CN-H_2O (3:1, 12 mL) was stirred at room temperature for 1 h.[3] The solution was diluted with water (50 mL) and extracted with $CHCl_3$ (3 x 50 mL). The organic phase was dried over Na_2SO_4 and evaporated. Flash chromatography afforded pure dicarbonyl compound 2-(2-oxo-ethyl)-benzaldehyde **1b** (8 mg, 27%) as colorless oil. R_f (petroleum ether/EtOAc 1:1) = 0.54; ^{1}H-NMR: 4.09 (s, 2H), 7.32-7.78 (m, 4H), 9.74 (s, 1H), 9.99 (s, 1H); ^{13}C-NMR: 48.2, 127.0, 132.8, 134.0, 135.6, 193.4, 198.3; m/z: 148, 147, 131, 120, 119, 91.

*trans-2-ethyl-3-phenyloxirane **4g***

Powdered *m*-CPBA (17 mmol, 70%) was added to a stirred heterogeneous mixture of Na_2HPO_4 (24 mmol) and alkene **1a** (12 mmol, 1.6 g) in CH_2Cl_2 at 0 °C within 30 minutes. The suspension was vigorously stirred at room temperature for 3 hours. $Na_2S_2O_5$ (18 mmol)

was added to destroy non reacted *m*-CPBA and the mixture was filtered. The filtrate was washed with aqueous saturated $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic phase was dried over Na_2SO_4 and finally evaporated. Flash chromatography afforded pure *trans*-2-ethyl-3-phenyloxirane **4** (1.36 g, 77%) as colorless liquid. R_f (petroleum ether/EtOAc 10:1) = 0.42; ^{1}H-NMR: 1.10 (t, 3H, *J*=7.5 Hz), 1.75 (m, 2H), 2.97 (dt, 1H, J_1=5.5 Hz, J_2=2.0 Hz), 3.65 (d, 1H, *J*=2.0 Hz), 7.28-7.40 (m, 5H); ^{13}C NMR: 9.48, 25.4, 58.3, 64.1, 125.5, 127.9, 128.4, 137.9

*1-phenylbutane-1,2-diol **3***

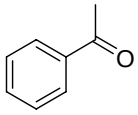
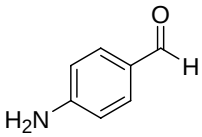
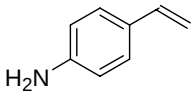
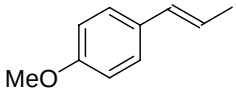
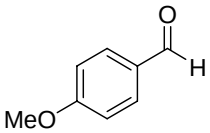
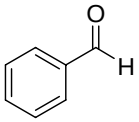
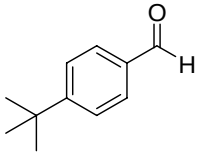
Concentrated H_2SO_4 was added to the epoxide *trans*-2-ethyl-3-phenyloxirane **4** (3.4 mmol, 0.5 g) stirred in THF/H_2O (2:3 v/v) at room temperature and the reaction was followed by TLC. NaCl was added until saturation was reached and the reaction mixture was extracted with EtOAc (3 x 20 mL). The organic layer was washed with $NaHCO_3$ (20 mL, sat., aq.) dried over Na_2SO_4 and finally evaporated. Flash chromatography (petroleum ether/EtOAc 10:1) gave a diastereomeric mixture of 1-phenylbutane-1,2-diol **3** as colorless liquid in 95% isolated yield (590 mg). R_f (petroleum ether/EtOAc 1:1) = 0.36; ^{1}H-NMR: 0.89-0.95 (m, 3H), 1.23-1.33 (m, 2H), 3.58-3.68 (m, 1H), 4.40-4.65 (m, 1H), 7.27-7.34 (m, 5H); ^{13}C-NMR: 9.9/10.2, 24.4/25.5, 77.3/77.6, 126.8, 127.7/127.9, 128.3/128.4, 140.5/141.3

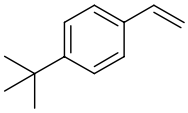
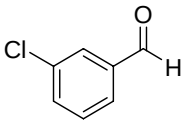
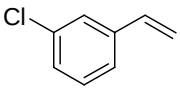
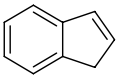
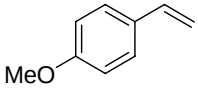
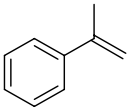
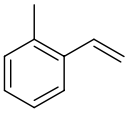
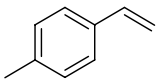
Indene oxide

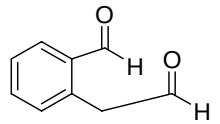
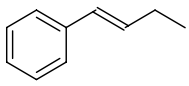
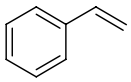
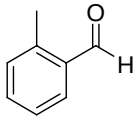
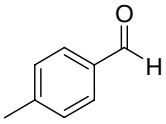
Powdered *m*-CPBA (1.03 mmol, 70%) was added to a stirred heterogeneous mixture of aqueous $NaHCO_3$ (0.3 N, 30 mL) and indene (0.86 mmol, 100 mg) at 0 °C over 15 min.[4] The suspension was vigorously stirred at room temperature for 0.5 hours and then extracted with ethyl ether (3 x 20 mL). The organic phase was washed with a cooled solution of 10% NaOH (20 mL), with brine (20 mL), dried over Na_2SO_4 and finally evaporated. Flash chromatography afforded indene oxide (30 mg, 27%) as colorless oil. R_f (petroleum ether/EtOAc 10:1) = 0.26; ^{1}H-NMR: 2.98 (dd, 1H, *J*=18.0 Hz), 3.21 (dd, 1H, *J*=18.0 Hz), 4.15 (dd, 1H, *J*=2.8 Hz), 4.29 (d, 1H, *J*=2.8 Hz), 7.26 (m, 3H), 7.51 (d, 1H, *J*=7.2 Hz); ^{13}C-NMR: 34.6, 57.6, 59.1, 125.1, 126.0, 126.2, 128.5, 140.8, 143.5

NMR-Data were confirmed by comparison with literature.[5]

Analytics

compound	GC-retention time [min]	GC-MS retention time [min]	GC-method/column	GC-MS method/column
 acetophenone	3.34	5.05	A	B
 *4*-aminobenzaldehyde	n.d.	7.30		B
 4-aminostyrene	n.d.	6.30		B
 *t*-anethole	4.44	6.61	A	B
 *p*-anisaldehyde	4.75	6.44	A	B
 benzaldehyde	2.97	4.03	A	B
 4-*tert*-butylbenzaldehyde	n.d.	6.76		B

compound	GC retention time [min]	GC-MS retention time [min]	GC-method/column	GC-MS method/column
 4-*tert*-butylstyrene	n.d.	6.23		B
 3-chlorobenzaldehyde	n.d.	5.53		B
 3-chlorostyrene	n.d.	5.11		B
 indene	2.91	4.80	A	B
 4-methoxystyrene	n.d.	5.77		B
 α-methylstyrene	2.54	4.23	A	B
 2-methylstyrene	n.d.	4.41		B
 4-methylstyrene	n.d.	4.43		B

compound	GC retention time [min]	GC-MS retention time [min]	GC-method/column	GC-MS method/column
 2-(2-oxo-ethyl)-benzaldehyde	5.52	6.90	A	B
 (*E*)-1-phenyl-1-butene	3.31	5.53	A	B
 styrene	n.d.	3.24		B
 *o*-tolualdehyde	n.d.	5.07		B
 *p*-tolualdehyde	n.d.	5.08		B

n.d. = not determined

Methods/Columns

A: 14.5 psi, 100 °C – 20 °C min^{-1} – 220 °C - hold 2 min.

GC-column: HP 1701 capillary column (50 m, 0.2 mm, 0.33 µm film)

B: 80 °C - hold 3 min – 30 °C min^{-1} – 250 °C - hold 10 min – 30 °C min^{-1} – 280 °C, 1 mL min^{-1}.

GC-MS column B: HP 5 MS capillary column (30 m, 0.25 mm, 0.25 µm film)

References

[1] J. S. Yadav, P. K. Deshpande, E. R. Reddy, *Synth. Commun.* **1989**, *19*, 125-134.

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