Supporting Information

From characterization to biocatalytic application of two peroxygenases from *Collariella virescens* and *Daldinia caldariorum*

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1. Gene sequences for Unspecific Peroxygenases from Collariella virescens and Daldinia caldariorum

Both Unspecific Peroxygenase genes were codon optimized for *E. coli* and cloned into a pET-28a expression plasmid under control of the T7 promoter. Both sequences contain an N-terminal 6HisTag which is underlined in the DNA sequence.

Gene sequence *Collariella virescens* Unspecific Peroxygenase (*Cvi*UPO)

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGAACTGGACTT
TAGTAAATGGAAGACCCGTCAGCCGGGCGAATTCCGTGCCCCGTGCCCGGCTATGAATTCTCTGGCCAACCACG
GTTTTATCCCGCGCGATGGCCGTAATATTACCGTAGCCATGCTGGTTCCGGTCCTGCAGGAGGTCTTCCACCTGT
CCCCAGAGCTGGCGCAGACGATCTCTACTCTGGGTCTGTTTACCGCTCAAGACCCTTCCAAAGGCGTATTCACTC
TAGACGACCTGAACCGCCATAACCTGTTTGAACATGATGCATCTCTGTCTCGTGAAGATTACTATTTCCACAAAGA
TGCATCTACCTTCCGTCCGGAAGTTTTCAAGAAGTTCATGTCCCACTTTAAAGGCAAGGAATATGTTACTCTGGA
AGACGCAGCTAGCGCCCGTTATGCAATGGTACAGGAAAGCCGCAAAAAAAAACCCGACTTTCACTTACACCGTTC
AGCAGCGTATCACCAGTTACGGTGAAACGATTAAATATTTCCGTACCATTGTTGAACCGGCTACTGGCAAGTGCC
CGGTTGCGTGGATTAAGATCCTGTTTGAACAGGAGCGACTGCCGTACAACGAGGGTTGGCGTCCGCCTAAAGCT
GAACTGTCTGGCTTTAGTATGGCATCCGATGTCCTGGAGCTGGCGTTAGTGACCCCGGAAAAACTGATCGACAA
ACCGTGTGAAGGCAAACAGTGCCCGCAAGCCCGCGGCATCCACGGTTACTTCGGCATGCTGCTGCCGATCACTG
CGCAGGAACTGGCAGTTAAGTAA

Gene sequence for *Daldinia caldariorum* Unspecific Peroxygenase (*Dca*UPO)

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGG
CACCGTGGAAAGCTCCTGGTCCGGACGACGTTCGCGGTCCGTGTCCAATGCTGAATACACTGGC
TAACCACGGTTTCCTGCCGCACGACGGTAAAAACATTGACGTTAATACCACCGTGAACGCTCTGT
CCTCCGCGCTGAACCTGGACGACGAATTATCCCGCGATCTGCACACCTTCGCAGTGACGACCAA
CCCGCAGCCGAACGCTACGTGGTTTAGCCTGAACCACTTATCCCGCCACAATGTGCTGGAACAC
GATGCATCTCTGTCCCGCCAGGATGCGTATTTCGGTCCACCTGATGTTTTCAACGCGGCTGTGTT
CAATGAAACCAAGGCTTATTGGACAGGCGACATCATTAACTTCCAGATGGCTGCGAACGCGTTGA
CCGCGCGTCTGATGACCTCCAACCTGACTAACCCGGAATTCTCTATGTCCCAGCTGGGTCGTGG
CTTCGGTCTGGGCGAAACTGTTGCTTATGTAACTATCCTGGGCTCTAAAGAAACACGCACTGTACC
GAAGGCGTTTGTTGAATACCTGTTCGAAAACGAACGTCTGCCGTACGAACTGGGTTTTAAAAAGA
TGAAATCTGCTCTGACTGAAGATGAACTGACTACCATGATGGGTGAAATTTATTCTCTGCAACACC
TGCCGGAAAGCTTTACCAAACCGTTCGCAAAACGTAGCGAAGCGCCGTTCGAAAAACGTGCGGA
AAAACGCTGCCCGTTCCACTAA

2. Analytical methods for GC and GC-MS analysis

GC analysis was performed on a Shimadzu GC-2010 Plus system with an AOC-20i autosampler, AOC-20s carrousel and a FID-2010 Plus detector. Different columns were used for analysis of different substrates (Table S1). GC-MS analysis was performed on a Shimadzu GC-2010 system with AOC-20i autosampler and a GCMS-GP2010S mass spectrometer detection system equipped with a Optima 1 (25m x 0.25 mm x 0.25 µm) column, see table S1 for temperature programs. Reactions were stopped by addition of ethyl acetate containing n-Dodecane (5mM) as internal standard. Reactions were subjected to liquid-liquid extraction and centrifugation after which the organic phase was subjected to GC(-MS) analysis.

Table S1. GC and GC-MS analytics. Linear velocity is 30 cm/sec, unless otherwise indicated.

Column	Temperature program / gradient	Retention time
GC CP wax 52 CB (Agilent) (25 m × 0.25 mm × 1.2 µm) carrier gas: N_2	120 °C 30 °C/min to 160°C, hold 5 min 30 °C/min to 245, hold 3 min	3.34 min n-Dodecane (IS) 3.45 min heptan-2-one 4.15 min heptan-2-ol 4.21 min octan-2-one 5.22 min octan-2-ol 5.79 min heptan-1-ol 7.53 min octan-1-ol
GC Hydrodex β-TBDM (MACHEREY-NAGEL) (50m x 0.25 mm x 0.15 µm) carrier gas: He	65 °C, hold 50 min 25 °C/min to 180 °C, hold 1 min 25 °C/min to 250 °C, hold 1 min Linear velocity : 38 cm / sec	20.9 min heptan-2-one 45.7 min R-heptan-2-ol[a] 46.7 min S-heptan-2-ol[a] 55.4 min n-Dodecane (IS)
GC CP-Chirasil-DEX CB (Agilent) (25 m × 0.32 mm × 0.25 µm) carrier gas: He	70 °C, hold 47.5 min 25 °C/min to 120 °C, hold 1 min 25 °C/min to 225 °C, hold 1 min	18.3 min 2-octanone 43.8 min R-octan-2-ol 44.7 min S-octan-2-ol 50.1 min n-Dodecane (IS)
GC CP wax 52 CB (Agilent) (25 m × 0.25 mm × 1.2 µm) carrier gas: N_2	120 °C, hold 5 min 30 °C/min to 140 °C, hold 1.5 min 30 °C/min to 180 °C, hold 1.5 min 30 °C/min to 245 °C, hold 1 min	4.67 min n-Dodecane (IS) 6.71 min oct-1-en-3-one 8.67 min oct-1-en-3-ol
GC CP-sil 8 CB (Agilent) (25m x 0.25mm x 1.2 µm) carrier gas: N_2	120 °C, hold 3 min 15 °C/min to 245 °C, hold 3.33 min 30 °C/min to 340 °C, hold 1 min	8.15 min n-Dodecane (IS) 8.94 min 4-phenylbutan-2-one 12.41 min rhododendrol
GC CP-Chirasil-DEX CB (Agilent) (25 m × 0.32 mm × 0.25 µm) carrier gas: He	120 °C, hold 2.6 min 15 °C/min to 135 °C, hold 3.3 min 25 °C/min to 225 °C, hold 1 min	2.38 min ethylbenzene 4.17 min acetophenone 4.59 min n-Dodecane (IS) 6.15 min R-1-phenylethan-1-ol 6.41 min S-1-phenylethan-1-ol
GC CP-sil 8 CB (Agilent) (25m x 0.25mm x 1.2 µm) carrier gas: N_2	120 °C, hold 3.5 min 30 °C/min to 180°C, hold 1 min 30 °C/min to 240 °C, hold 2 min 30 °C/min to 340, hold 1 min	3.15 min toluene 5.27 min pseudocumene 6.06 min p-cymene 7.82 min n-Dodecane (IS) 8.72 min thymol
GC CP-sil 8 CB (Agilent) (25m x 0.25mm x 1.2 µm) carrier gas: N_2	110 °C, hold 3 min 30 °C/min to 160 °C, hold 8.33 min 30 °C/min to 340 °C, hold 1 min	5.26 min styrene 6.42 min alpha methyl styrene 6.70 min cis-beta-methyl styrene 7.18 min trans-beta-methyl styrene 7.57 min phenyl acetaldehyde 8.10 min styrene oxide

		8.42 min alpha methyl styrene oxide 8.71 min phenyl propanal 8.88 min cis-beta-methyl styrene oxide 9.12 min trans beta-methyl styrene oxide 9.41 min phenyl acetone 10.9 min n-Dodecane (IS) 13.91 min cinnamaldehyde 14.42 min phenyl-1,2-propanediol 14.51 min cinnamyl alcohol
GC CP-sil 8 CB (Agilent) (25m x 0.25mm x 1.2 µm) carrier gas: N_2	90 °C, hold 3 min 25 °C/min to 289 °C, hold 2 min 25 °C/min to 340 °C, hold 1 min	6.34 min alpha pinene 7.30 min limonene 7.48 min n-Dodecane (IS) 8.08 min alpha pinene oxide 8.24 min camphenol 8.29 min cis- limonene oxide 8.42 min verbenol 9.67 min limonene dioxide 10.9 min beta-ionone 11.6 min 7,11-Epoxymegastigma-5(6)-en-9-one 12.0 min 4-Hydroxyy Beta Ionone
GC CP-sil 8 CB (Agilent) (25m x 0.25mm x 1.2 µm) carrier gas: N_2	180 °C, hold 5 min 30 °C/min to 280 °C, hold 5 min 30 °C/min to 340 °C, hold 1 min	3.37 min thioanisole 4.16 min n-Dodecane (IS) 4.50 min methyl p-tolyl sulfide 6.23 min methyl phenyl sulfoxide 6.83 min methyl phenyl sulfone 7.52 min methyl p-tolyl sulfoxide 8.03 min methyl p-tolyl sulfone
GC-MS Optima 1 (MACHEREY-NAGEL) (25m x 0.25mm x 0.25 µm) Carrier gas: He	120 °C, hold 7 min 30 °C/min to 180 °C, hold 1 min 30 °C/min to 240 °C, hold 2 min 30 °C/min to 340 °C, hold 1 min MS detection started after 3 min	5.35 min n-Dodecane (IS) 5.61 min thymohydroquinone 5.97 min thymol

[a] Chiral reference compounds were unavailable for this substrate. Identification of R- and S- elution times are based on observation that for both octan-2-ol and 1-phenylethan-1-ol the R-enantiomer elutes first.

3. Chiral analysis of alcohol oxidation

Chromatograms of chiral analysis of enantiomers of 2-octanol and 1-phenylethanol. The top chromatogram shows chiral analysis of the enantiomers of octan-2-ol. In the top chromatogram, the pink line shows the R- and S-enantiomer peaks of octan-2-ol at t=0 of the enzymatic reaction. The blue line shows remaining peaks to the R- and S-enantiomers of octan-2-ol after a reaction time of 3 hours with the DcaUPO. The black line in the chromatogram shows the peak for R-octan-2-ol as authentic standard. A slight preference for the consumption of R-octan-2-ol can be observed. The bottom chromatogram shows the chiral analysis of 1-phenyletahnol. The blue line in this chromatogram shows the starting point of both enantiomers at t=0 in the enzymatic reaction. The black line shows the remaining peaks of the R- and S-enantiomers of 1-phenylethanol after a reaction time of 3 hours with the *Cci*UPO whereas the pink line shows the remaining peaks of the R- and S-enantiomers of 1-phenylethanol after a reaction time of 3 hours with the *Dca*UPO. A slight preference for the consumption of the R-enantiomer is observed for the *Dca*UPO.

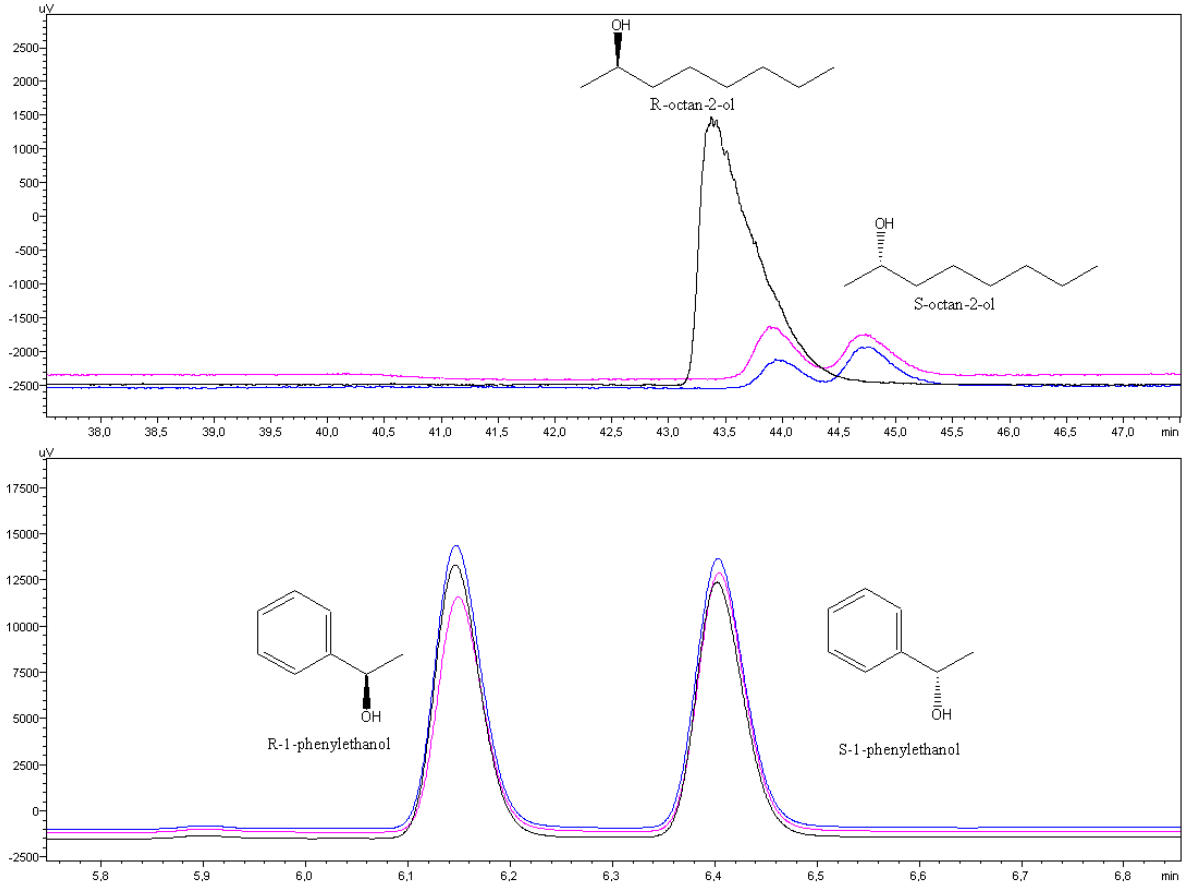

Figure S1: chromatograms of chiral GC analysis of octan-2-ol (top) and 1-phenylethanol (bottom). In the top chromatogram, the pink line shows the starting point of both the R- and S- enantiomers for octan-2-ol. The blue line shows the remaining R- and S-enantiomers after a reaction time of 3hours with *Dca*UPO. Black line shows authentic standard for R-octan-2-ol. In the bottom chromatogram, the blue line shows the starting points of both the R- and S-enantiomer of 1-phenylethanol. The black line shows the remaining R- and S-enantiomer peaks after a reaction time of 3 hours with the *Cci*UPO, the pink line shows the remaining R- and S-enantiomer peaks after a reaction time of 3 hours with the *Dca*UPO.

4. GC-MS spectra of thymol, pseudocumene and products

GC-MS spectra of thymol and product (Figure S2) compared to reference GS-MS spectra and GC-MS spectra of pseudocumene and product (Figure S3) compared to reference GC-MS spectra.

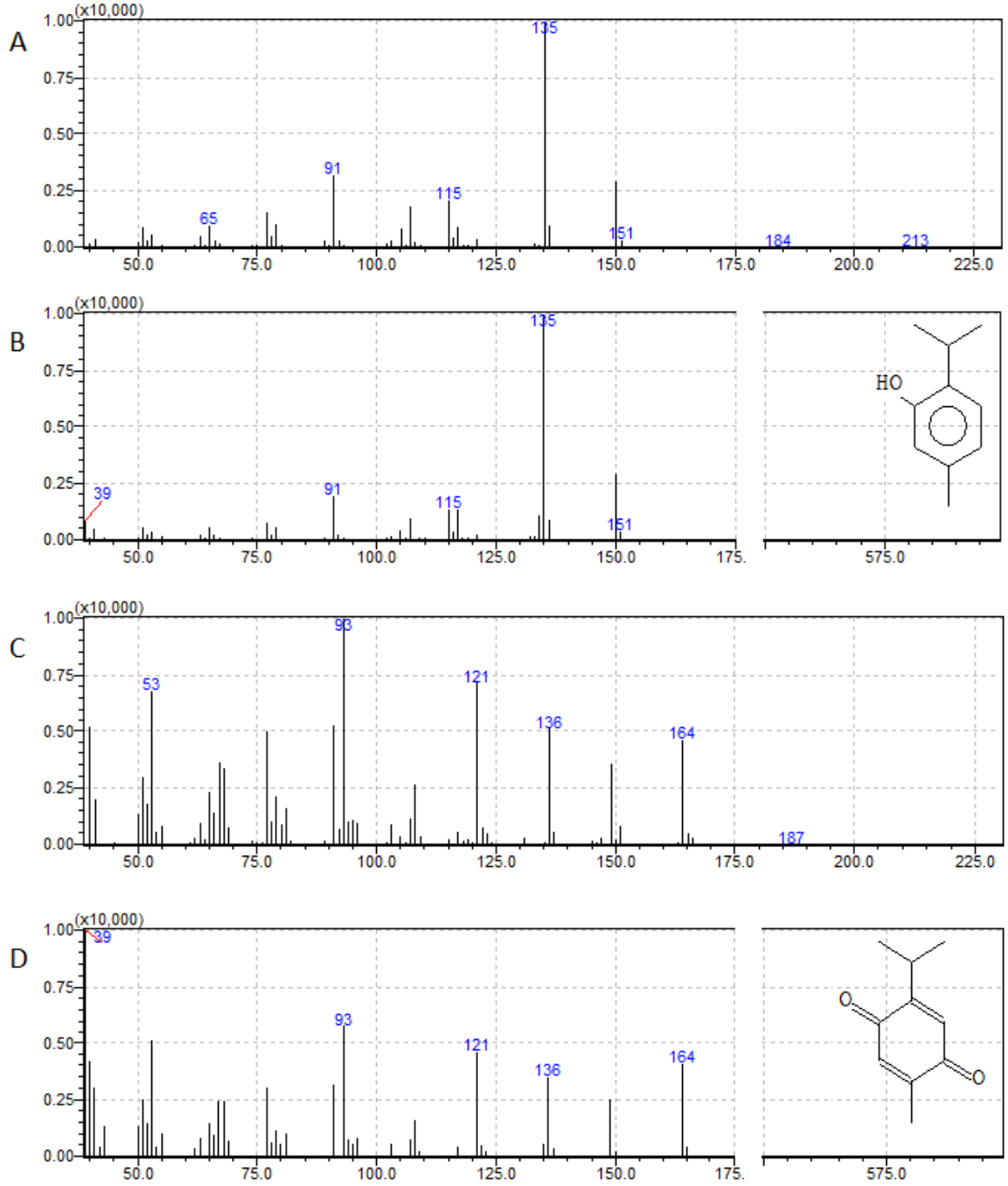

Figure S2: GC-MS spectra of thymol, the observed reaction product and comparisons to reference GC-MS spectra. A: GC-MS spectra of the substrate thymol, B: GC-MS reference spectra of thymol, C: GC-MS spectra of the observed product of the reaction with *Dca*UPO, D: Reference GC-MS spectrum of the presumed product, thymoquinone.

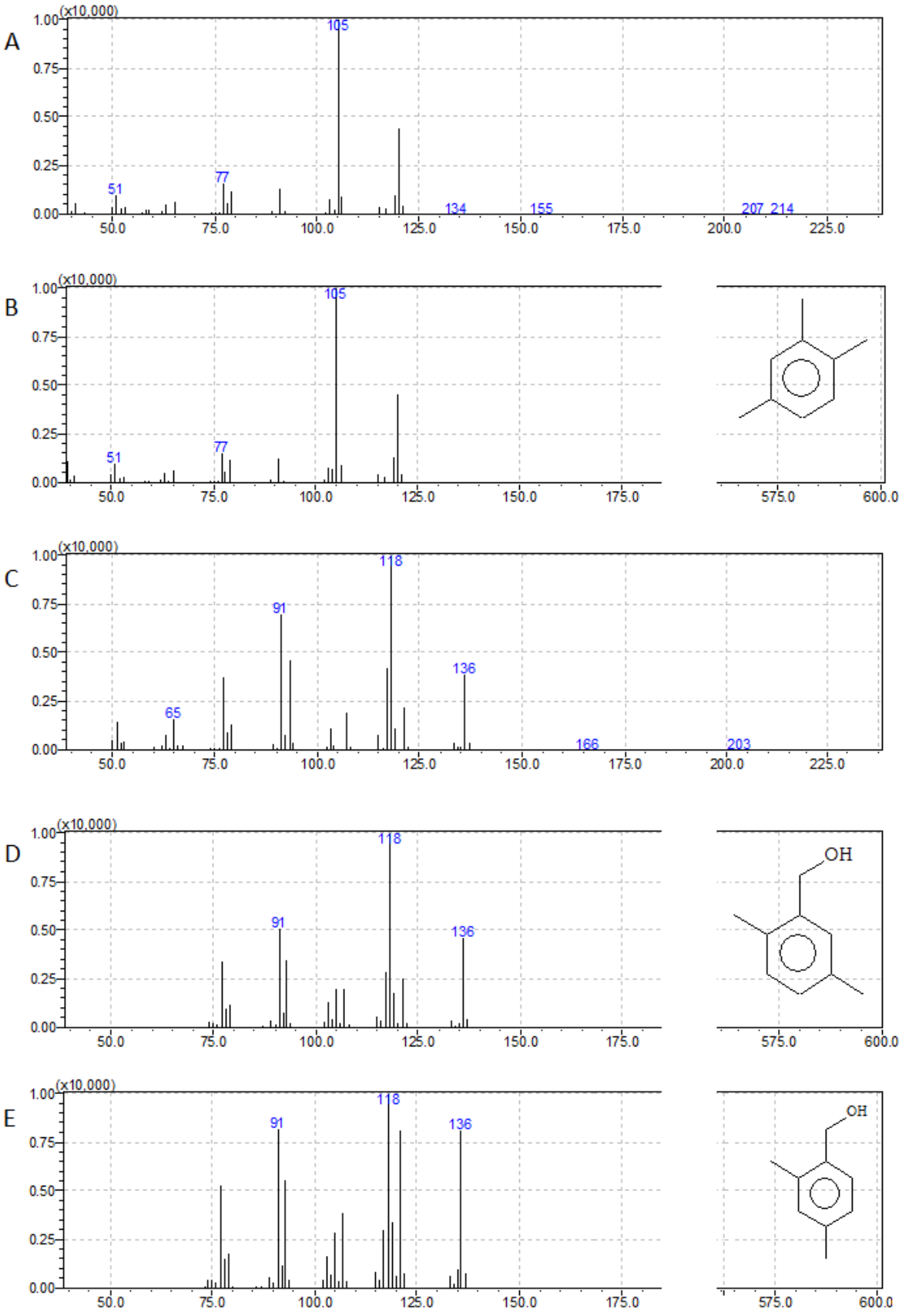

Figure S3: GC-MS spectra of pseudocumene, the observed reaction product and reference spectra of potential products. A: GC-MS spectra of the substrate pseudocumene, B: GC-MS reference spectra of pseudocumene, C: GC-MS spectra of the observed product of the reaction with *Dca*UPO, D and E: Reference GC-MS spectra of the potential products.