Regioselective *ortho*-Carboxylation of Phenols Catalyzed by Benzoic Acid Decarboxylases: a Biocatalytic Equivalent to the Kolbe-Schmitt Reaction

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Electronic Supplementary Information

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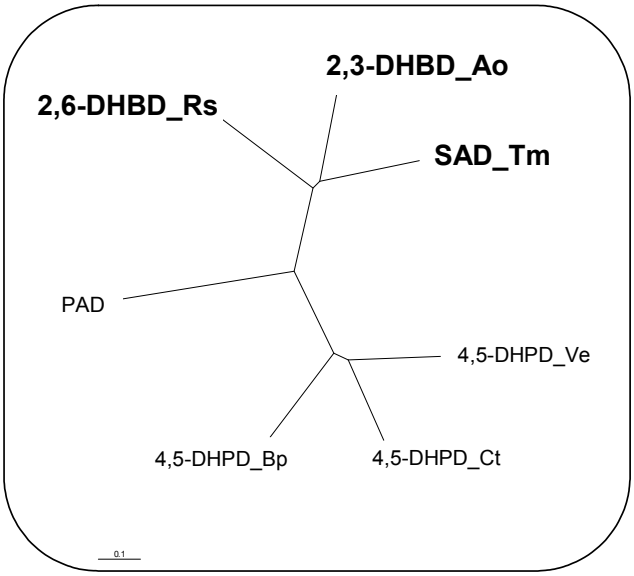


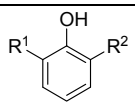
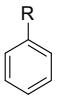
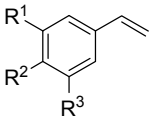
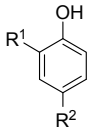
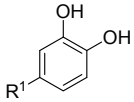
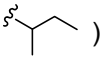
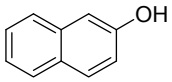
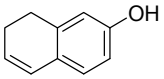
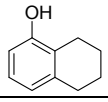
Fig. S1 Phylogenetic tree of benzoic/phthalic acid decarboxylases (DHB/PDs) and salicylic acid decarboxylase (SAD) in relation to phenolic acid decarboxylase from *Lactobacillus plantarum* (PAD).

Table S1 Sequence relationship between enzyme candidates[a]

Entry	Enzyme	Similarity [%]	Identity [%]
1	2,3-DHBD_Ao vs 2,6-DHBD_Rs	80	66
2	2,6-DHBD_Rs vs SAD_Tm	62	45
3	SAD_Tm vs 2,3-DHBD_Ao	88	77

[a] For amino acid sequences see C. Wuensch, S. M. Glueck, J. Gross, D. Koszelewski, M. Schober, K. Faber, *Org. Lett.* 2012, **14**, 1974–1977.

Table S2 Non-substrates for recombinant benzoic acid decarboxylases 2,3-DHBD_Ao, 2,6-DHBD_Rs and SAD_Tm.

Entry	Non-Substrates (**32a-49a**) Conversion <1%		HPLC method[a]	Retention time [min]
1		**32a** ($R^1 = R^2 = OMe$)	B	22.9
2		**33a** ($R = NH_2$)	G	14.7
3		**34a** (R = SH)	B	29.9
4		**35a** ($R^1 = R^2 = R^3 = H$)	B[b]	32.5
5		**36a** ($R^2 = Cl$, $R^1 = R^3 = H$)	B[b]	34.5
6		**37a** ($R^2 = OMe$, $R^1 = R^3 = H$)	B[b]	32.0
7		**38a** ($R^2 = NH_2$, $R^1 = R^3 = H$)	B	18.2
8		**39a**	B	14.7
9		**40a** ($R^1 = NO_2$, $R^2 = H$)	B	27.2
10		**41a** ($R^1 = H$, $R^2 = NO_2$)	F	13.1
11		**42a** ($R^1 = CH{=}O$, $R^2 = H$)	B	25.1
12		**43a** (R = *n*-propyl)	B	30.0
13		**44a** (R = *iso*-propyl)	B	28.3
14		**45a** ($R^1 = CH_3$)	F	11.8
15		**46a** (R^1 = )	F	14.6
16		**47a**	B	27.3
17		**48a**	B	28.4
18		**49a**	F	14.1

[a] Column: Phenomenex Luna (PL), C18 (2) 100 Å, 250 x 4.6 mm, 5 µm, column temperature 24 °C; method **B** was run over 35 min with H_2O/trifluoroacetic acid (0.1%) as the mobile phase (flow rate 0.5 mL/min) and an acetonitrile/trifluoroacetic acid (0.1%) gradient (0-2 min 0%, 2-30 min 0-100%, 30-35 min 100%); [b] method B was maintained for 40 min; method **F** was run over 17 min with H_2O/trifluoroacetic acid (0.1%) as the mobile phase (flow rate 1 mL/min) and an acetonitrile/trifluoroacetic acid (0.1%) gradient (0-2 min 0%, 2-15 min 0-100%, 15-17 min 100%); method **G** was run over 25 min with H_2O/trifluoroacetic acid (0.1%) as the mobile phase (flow rate 0.5 mL/min) and an acetonitrile/trifluoroacetic acid (0.1%) gradient (0-2 min 0%, 2-14 min 0-47%, 15-20 min 47-100%, 20-25 min 100%).

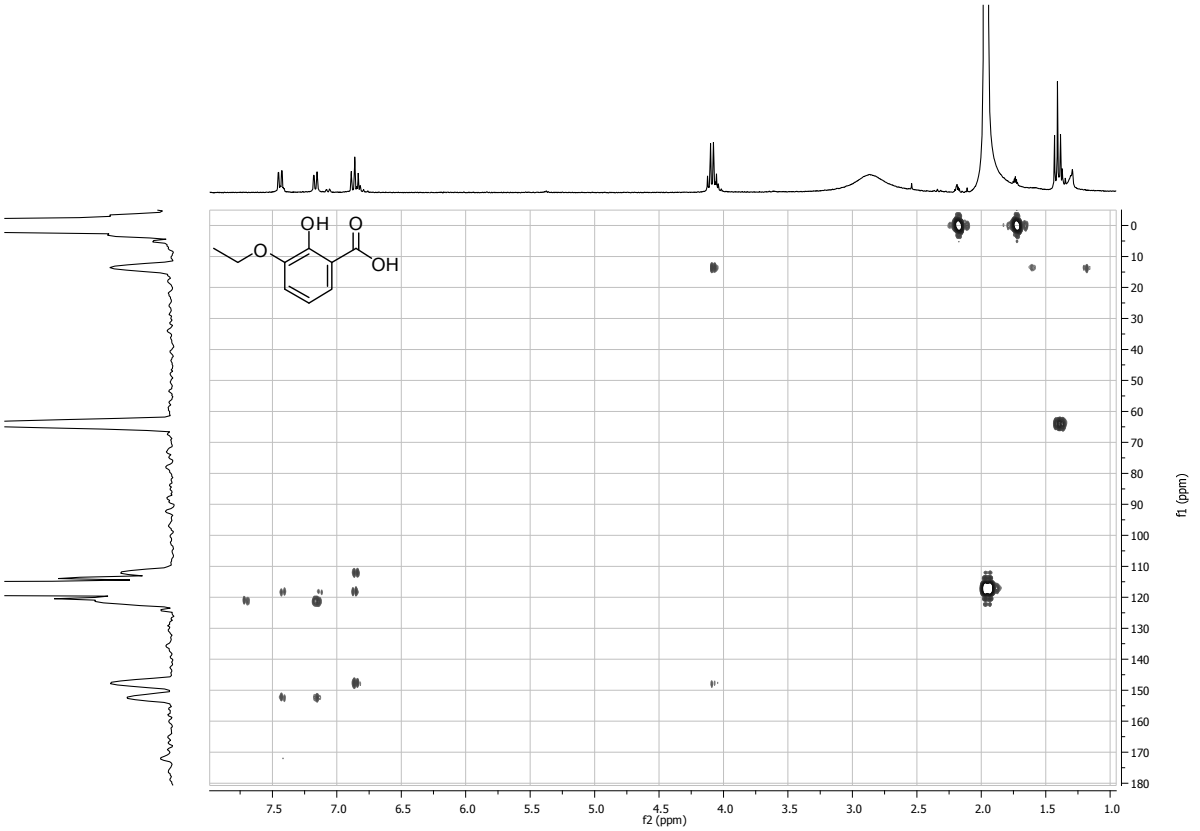
Table S3 HPLC analysis of substrates (**1a-31a**) and products (**1b-31b**[a])

Substrate	Product	Column[b]	HPLC method	Retention time [min]	
				Substrate	Product
1a	**1b**	PL	B	25.7	27.1
2a	**2b**	PL	B	25.2	26.3
3a	**3b**	PL	E	10.4	10.7
4a	**4b**	PL	B	23.6	22.9
5a	**5b**	PL	A	30.1	32.7
6a	**6b**	PL	E	9.3	10.1
7a	**7a**	PL	B	26.3	25.0
8a	**8b**	PL	B	25.8	27.3
9a	**9b**	PL	B	25.8	26.8
10a	**10b**	PL	B	26.6	27.9
11a	**11b**	PL	F	14.1	14.4
12a	**12b**	PL	B	26.5	27.7
13a	**13b**	PL	E	11.2	11.5
14a	**14b**	PL	F	14.4	14.7
15a	**15b**	MNN	C	4.5	5.8
16a	**16b**	PL	H	5.3	45.9
17a	**17b**	PL	H	4.2	6.8
18a	**18b**	PL	A	21.8	23.4
19a	**19b/19c**[a]	PL	G	3.2	3.6/3.4
20a	**20b**	PL	B	15.9	19.8
21a	**21b**	PL	B	20.3	23.7
22a	**22b**	PL	B	28.0	32.4
23a	**23b**	PL	B	22.9	24.2
24a	**23b**	PL	F	10.1	10.8
25a	**25b**	PL	F	12.3	12.7
26a	**26b**	PL	B	14.4	18.1
27a	**27b**	MNN	D	4.5	5.9
28a	**28b**	PL	B	28.1	29.2
29a	**29b**	PL	B	26.3	27.4
30a	**30b**	PL	B	26.8	25.7
31a	**31b**	PL	B	27.9	26.9

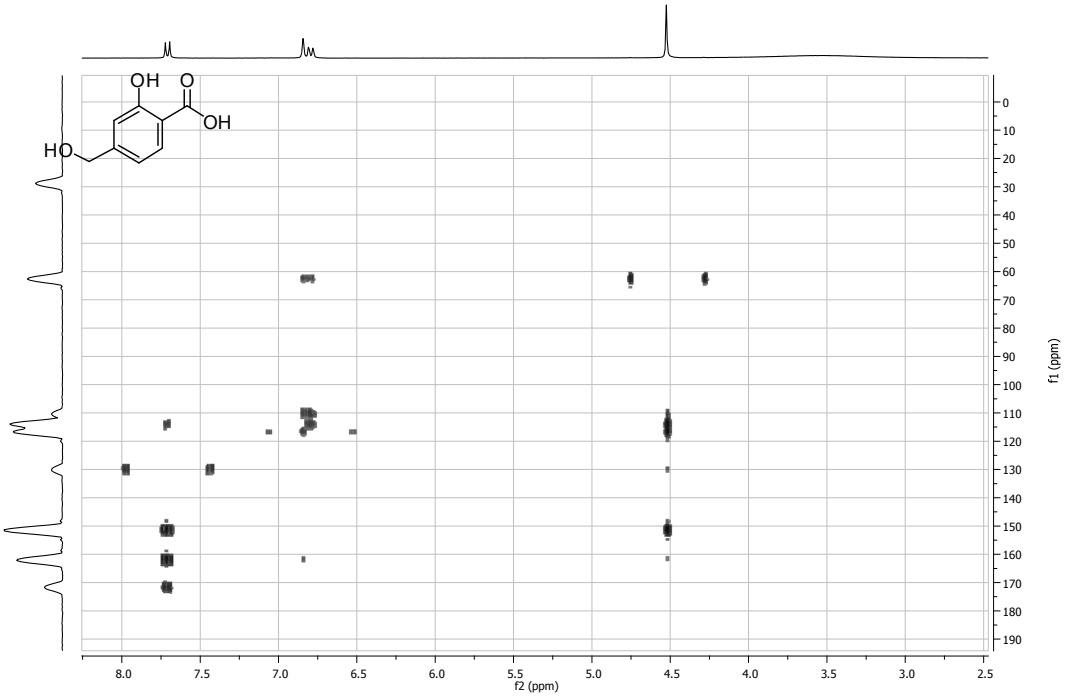
[a] In case of substrate **19a** two regioisomers were obtained as products: 2,6- (**19b**) and 2,4- (**19c**) dihydroxybenzoic acid; [b] columns: Phenomenex Luna (PL); Macherey Nagel Nucleodur (MNN);

HMBC-NMR Spectra

3-Ethoxysalicylic acid (7b):



2-Hydroxy-4-(hydroxymethyl)benzoic acid (24b):



5-Allyl-2-hydroxy-3-methoxybenzoic acid (31b):