Supplementary Material for Organic & Biomolecular Chemistry

Supplementary Information for

Biosynthesis of *iso*-fatty acids in myxobacteria

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1. Results of fatty acid analysis

Table 1 Fatty acid profile of *Stigmatella aurantiaca* wild-type strain (WT) and a *bkd* mutant (*bkd*) without any feeding, and of the *bkd* mutant after feeding of isobutyric acid (IBA) and isovaleric acid (IVA). Amounts are given in % of total area in GC.

Compound	WT		*bkd* IBA	IVA
iso-11:0	0.4	-	-	-
iso-13:0	0.4	-	-	-
iso-14:0	0.4	1.9	-	-
iso-15:1	0.9	0.7	1.4	1.4
iso-15:0	22.3	11.9	17.3	24.3
iso-15:0 3OH	2.0	1.4	2.3	1.9
iso-16:1	-	1.2	-	-
iso-16:0	5.4	3.1	10.9	5.1
iso-17:1 (*ω*–10 *cis*)	-	2.2	1.2	-
iso-17:1 (*ω*–5 *cis*)	1.4	0.8	8.1	-
iso-17:0	11.9	5.0	-	7.7
iso-17:0 3OH	2.0	2.2	2.9	-
10:0	-	-	-	-
14:1 (*ω*–10 *cis*)	0.3	-	-	-
14:0	1.5	-	1.0	1.0
15:1 (*ω*–10 *cis*)	0.3	-	-	-
15:1 (*ω*–5 *cis*)	1.3	0.8	1.2	0.9
16:1 (*ω*–5 *cis*)	24.0	32.4	26.1	24.3
16:0	2.7	8.7	1.7	1.5
16:0 3OH	-	0.9	-	0.9
16:0 2OH	0.5	2.5	1.1	0.8
17:0	0.5	-	-	-
18:1	10.5	10.7	10.5	11.1
18:0	0.6	1.0	-	-

Table 2 Fatty acid profile of *Myxococcus xanthus* wild-type strain (WT) and a *bkd* mutant (*bkd*) without any feeding, and of the *bkd* mutant after feeding of isobutyric acid (IBA) and isovaleric acid (IVA). Amounts are given in % of total area in GC.

Compound	WT		*bkd*	
			IBA	IVA
iso-13:0	0.3	-	-	-
iso-14:0	-	0.3	1.1	-
iso-15:1	0.5	-	-	0.9
iso-15:1	0.3	0.4	0.5	1.4
iso-15:0	37.4	14.6	10.9	34.0
iso-15:0 3OH	1.1	0.3	-	0.5
iso-16:1	-	1.0	5.7	-
iso-16:1	-	0.5	0.7	-
iso-16:0	-	4.9	12.1	1.3
iso-17:2	2.3	0.5	0.5	1.8
iso-17:1 (*ω*–10 *cis*)	1.1	0.5	0.4	1.1
iso-17:1 (*ω*–5 *cis*)	2.4	1.6	1.3	2.8
iso-17:0	3.6	4.1	2.2	4.3
iso-17:0 2OH	1.4	0.5	0.5	0.6
iso-17:0 3OH	1.2	0.5	0.5	0.5
8:0 3OH	0.3	-	-	0.3
10:0	0.5	-	0.2	0.7
13:1	0.2	1.0	0.9	0.6
13:0	0.5	0.4	-	-
14:1 (*ω*–10 *cis*)	1.0	0.7	0.9	0.9
14:1 (*ω*–5 *cis*)	-	0.4	0.4	0.3
14:0	4.2	3.3	3.0	2.9
15:1 (*ω*–10 *cis*)	3.4	5.0	4.4	3.0
15:1 (*ω*–5 *cis*)	3.1	5.0	5.6	3.4
15:0	4.0	9.2	7.7	2.9
16:2 (*ω*–5 cis, *ω*–11 *cis*)	4.5	4.4	4.7	4.7
16:1 (*ω*–11 *cis*)	1.2	2.1	1.3	1.3
16:1 (*ω*–5 *cis*)	13.8	27.6	25.3	17.5
16:0	1.6	3.3	2.5	1.3
16:0 3OH	0.3	0.3	0.5	-
16:0 2OH	-	-	-	-
17:1 (*ω*–10 *cis*)	-	0.8	0.6	-
17:1 (*ω*–5 *cis*)	-	0.4	0.5	-
17:0	-	0.6	0.5	-

2. Identification of methyl-branched fatty acid methyl esters by a retention index increment system

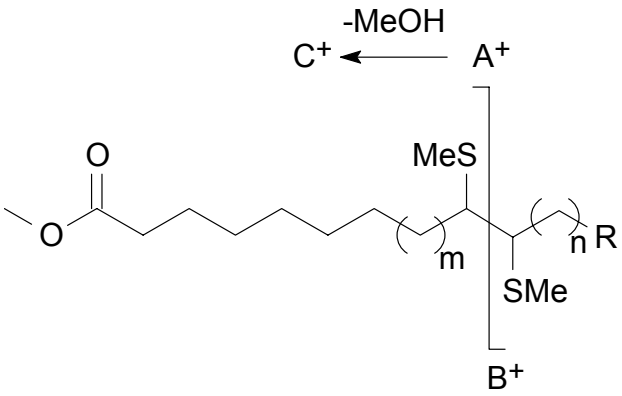
Branched chain fatty acid methyl esters (FAMEs) have lower retention times in GC compared to their unbranched counterparts. The position of a methyl branch can be derived from the retention index of the methyl ester. Calculated retention indices I_C of methyl-branched compounds with various functional groups (e. g. alkanes,[1] 3-ketones or 3-alcohols[2]) can be predicted by an increment system according to Equation 1.

$$I_C = N + FG + Me_i + S \quad (1)$$

Herein N is the number of carbon atoms in the longest chain times one hundred, FG is a functional group increment, that is FG(ME) = 332 for methyl esters (BPX-5 fused silica capillary column) and Me_i is an increment for the methyl group depending on its position. The different values for Me_i are given in the literature,[1] and it is Me_i = 60 for a methyl branch in *ω*–1 position. A steric increment S only has to be taken into account in case of multi methyl-branched compounds.

3. Identification of unsaturated fatty acids by derivatization with dimethyl disulfide (DMDS)

The position of double bonds in unsaturated fatty acids cannot be derived from mass spectra, because the mass spectra of unsaturated fatty acid methyl esters only differing in the position of the double bond are very similar. The double bond position can be determined by derivatisation with dimethyl disulfide giving adducts as shown in Figure 1. These DMDS adducts show strong fragmentation between the two methylthio groups leading to the fragment ions A^+ and B^+. Furthermore, the ion containing the methyl ester function is characterised by the loss of methanol giving ion C^+. The mass spectra of DMDS adducts obtained from *Stigmatella aurantiaca* after feeding with [D_7]-15-methylhexadecanoic acid are shown in Figure 2.



14:1 (ω-5 *cis*): m=1, n=3, R=Me
16:1 (ω-5 *cis*): m=3, n=3, R=Me
[D_7]*iso* 17:1 (ω-7 *cis*): m=1, n=4, R=[D_7]-*i*Pr
[D_7]*iso* 17:1 (ω-6 *cis*): m=2, n=3, R=[D_7]-*i*Pr
[D_7]*iso* 17:1 (ω-5 *cis*): m=3, n=2, R=[D_7]-*i*Pr
[D_7]*iso* 17:1 (ω-4 *cis*): m=4, n=1, R=[D_7]-*i*Pr

Fig. 1 Fragmentation of DMDS adducts of unsaturated fatty acid methyl esters in MS.

Table 3 DMDS adducts obtained from a methylated fatty acid extract of *Stigmatella aurantiaca* fed with [D_7]-15-methylhexadecanoate. M^+: molecular ion, A^+, B^+, C^+: fragment ions in Figure 1.

Parent Compound	M^+	A^+	B^+	C^+
14:1 (ω-5 *cis*)	334	217	117	185
16:1 (ω-5 *cis*)	362	245	117	213
[D_7]*iso*-17:1 (ω-7 *cis*)	383	217	166	185
[D_7]*iso*-17:1 (ω-6 *cis*) [a]	383	231	152	199
[D_7]*iso*-17:1 (ω-5 *cis*) [a]	383	245	138	213
[D_7]*iso*-17:1 (ω-4 *cis*) [a]	383	259	124	227

[a] DMDS derivatives coelute in GC (compare Figure 2D).

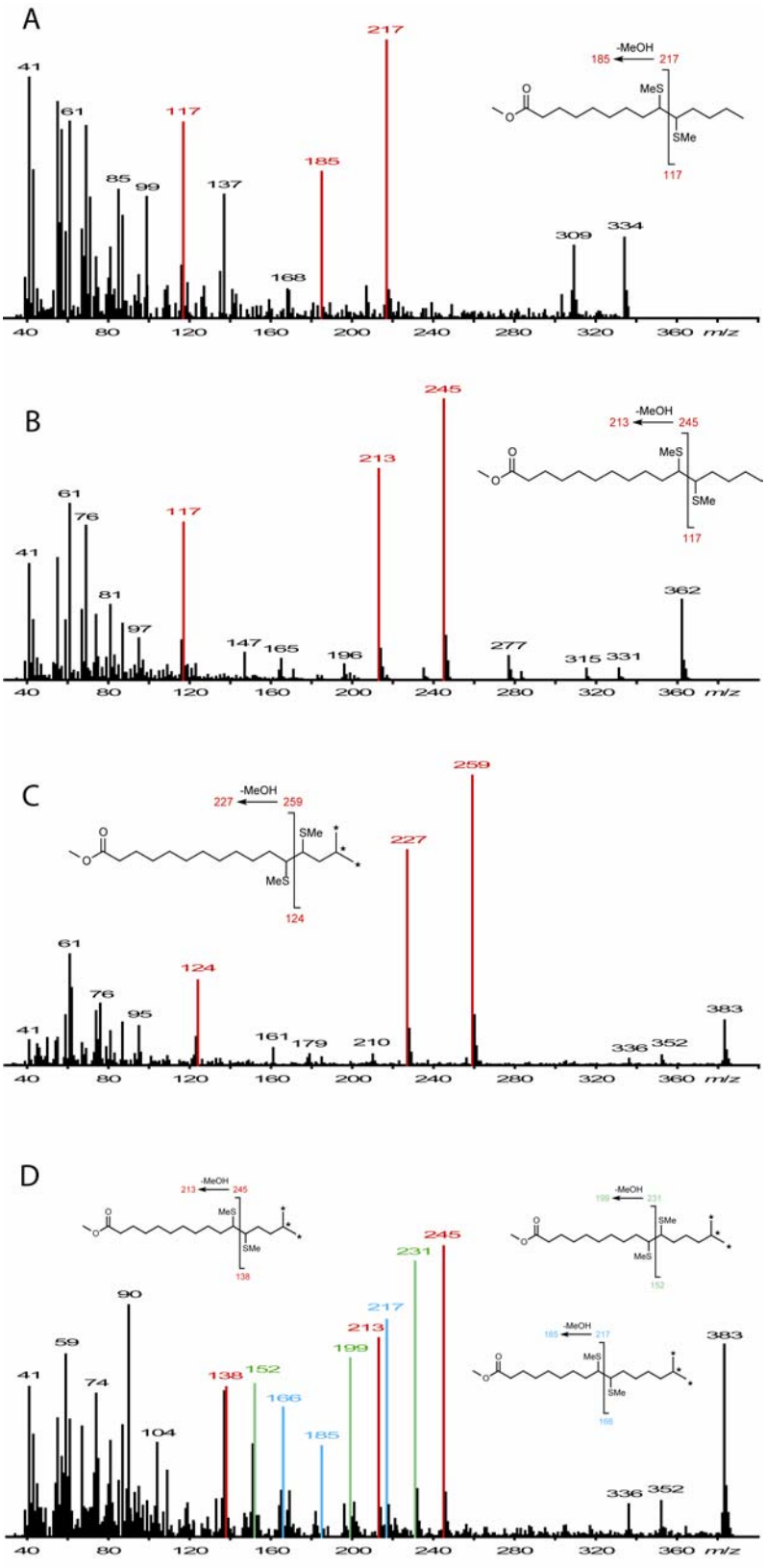


Fig. 2 DMDS adducts of unsaturated fatty acids obtained from *Stigmatella aurantiaca* after feeding with [D_7]-15-methylhexadecanoic acid. (A) 14:1 (*ω*-5, *cis*), (B) 16:1 (*ω*-5, *cis*), (C) [D_7]*iso*-17:0 (*ω*-4, *cis*), (D) coeluting [D_7]*iso*-17:0 (*ω*-5, *cis*), [D_7]*iso*-17:0 (*ω*-6, *cis*), and [D_7]*iso*-17:0 (*ω*-7, *cis*).

References

1 S. Schulz, *Lipids*, 2001, **36**, 637-647.

2 J. S. Dickschat, S. C. Wenzel, H. B. Bode, R. Müller, S. Schulz, *ChemBioChem*, 2004, **4**, 778-787.