Biosynthesis of 15,16-Dimethyltriacontanedioic Acid (Diabolic Acid) from [16-$^2H_3$]- and [14-$^2H_2$]-Palmitic Acids

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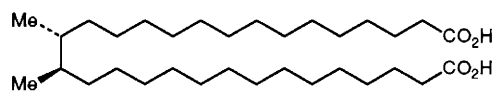
It is shown that no loss of deuterium occurs during formation of 15,16-dimethyltriacontanedioic acid (diabolic acid) from [16-$^2H_3$]palmitic acid and [14-$^2H_2$]palmitic acid in *Butyrivibrio fibrisolvens*.

Diabolic acid **1** [(15*R*,16*R*)-15,16-dimethyltriacontanedioic acid†] has been identified as a major component in the complex lipids of *Butyrivibrio fibrisolvens*.[1,2] The same compound was later detected as a lipid constituent in *Thermotoga maritima*.[3] The auxotrophic organism *B. fibrisolvens* str.S2 has been shown to grow only if supplied with free long-chain fatty acids or complex lipids, which can be transformed into fatty acids by lipolysis.[4] Growth is best stimulated by saturated straight-chain fatty acids (C_{13}–C_{18}), which give the corresponding long-chain dicarboxylic acids containing *vicinal* dimethyl branching.[1,4] Thus, this organism is ideally suited for incorporation experiments with stable isotopes since its fatty acid auxotrophy avoids endogenous dilution.

To probe the mechanism of the unusual coupling reaction, which generates diabolic acid **1** from palmitic acid, we have carried out two feeding experiments with commercially available [16-$^2H_3$]palmitic acid **2**, respectively with [14-$^2H_2$] palmitic acid **3** which was synthesized as shown in Scheme 1.

For incubation experiments six 1 l batches of fatty acid free culture medium[4] supplemented with 30 mg l^{-1} of labelled precursor were inoculated with six 100 ml cultures of *B. fibrisolvens* str.S2, which had been cultured in the same medium for 20 h at 39 °C under CO_2. After 24 h under these same conditions the cells were harvested by centrifugation and extracted with $CHCl_3$–MeOH (2 : 1).[5] Lipid transesterification with HCl–MeOH by a standard method[6] gave 105 mg of a mixture from which 35 mg of pure dimethyl 15,16-dimethyltriacontanedioate were isolated by chromatography on a flash silica gel column (hexane–diethyl ether 10 : 1, R_f = 0.26). The specimens of the dimethyl esters from the two feeding experiments were analysed for deuterium content by mass



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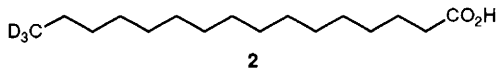

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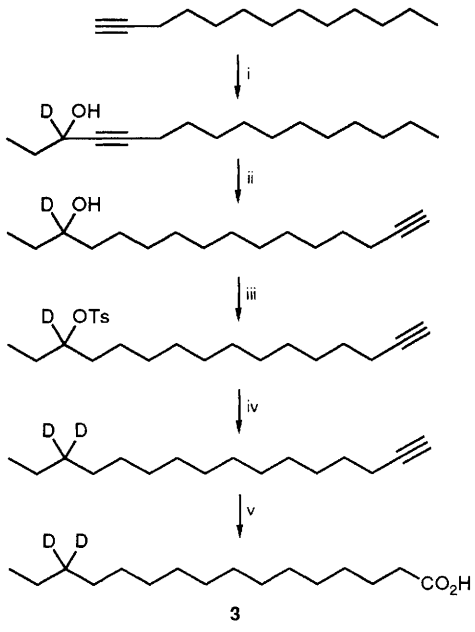
† The (15*R*,16*R*)-configuration indicated in **1** was determined by synthesis of the natural enantiomer from (2*S*,3*S*)-2,3-dimethylsuccinic acid.

Table 1 Incorporation experiments

Experiment	Deuterium distribution[a] Precursor	Diabolic acid **1**		
1	[16-$^2H_3$]palmitic acid **2** 1.5 ± 0.1% [$^2H_2$] 98.3 ± 0.2% [$^2H_3$]	1.6 ± 0.2% [$^2H_4$]	3.1 ± 0.3% [$^2H_5$]	95.2 ± 0.7% [$^2H_6$]
		(calc.[b] 0.3 ± 0.2% [$^2H_4$]	3.0 ± 0.2% [$^2H_5$]	96.6 ± 0.4% [$^2H_6$])
2	[14-$^2H_2$]palmitic acid **3** 5.1 ± 0.1% [$^2H_1$] 94.8 ± 0.1% [$^2H_2$]	3.3 ± 0.8% [$^2H_3$]	9.2 ± 0.6% [$^2H_2$]	87.4 ± 1.0% [$^2H_4$]
		(calc.[b] 0.4 ± 0.2% [$^2H_2$]	9.7 ± 0.2% [$^2H_3$]	89.9 ± 0.2% [$^2H_4$])

[a] Established by evaluation of the mass spectra of the corresponding methyl esters. The values correspond to the average of at least three independent measurements. [b] Calculated for no loss of deuterium.



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Scheme 1 Ts = *p*-$MeC_6H_4SO_2$ *Reagents and conditions*: i, Bu^nLi (1 equiv.), then Et-CDO (≥97% D, 1 equiv.), tetrahydrofuran, −65 °C, 79%; ii, potassium 3-aminopropylamide (KAPA) (4 equiv.), 3-aminopropylamine, room temp., 78%; iii toluene-*p*-sulfonyl chloride (2 equiv.), pyridine, room temp., 73%; iv, lithium triethylborodeuteride (3 equiv.), tetrahydrofuran, room temp., 82%; v, Bu^nLi (1 equiv.), trimethylchlorosilane (1 equiv.), tetrahydrofuran, −40 °C, then dicyclohexylborane (1.1 equiv.), tetrahydrofuran, 0 °C, then hydrogen peroxide (5 equiv.), MeOH–2 mol dm^{-3} NaOH (5:1), 50 °C, 64%

spectrometry. The results are summarized in Table 1; comparison of the experimental values with the calculated ones provides evidence that the two precursors are incorporated into diabolic acid **1** without loss of label and this, in turn, rules out the intermediacy of Δ^{14}- or Δ^{15}- unsaturated fatty acids or derivatives thereof.

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