# Stereochemical Course of Dehydration Catalysed by the Yeast Fatty Acid Synthetase

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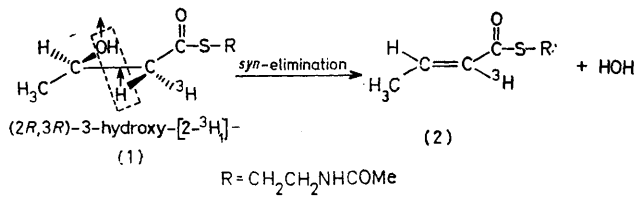
*Summary* Dehydration of the (3*R*)-hydroxyalkyl *S*-thioester (**1**) by yeast fatty acid synthetase to give the *trans*-2-enoyl derivative (**2**) occurs by means of a *syn*-elimination of the elements of water.

THE biosynthesis *de novo* of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA involves a cyclic series of condensation–reduction–dehydration–reduction reactions that result in the lengthening of the acyl-chain by one $-CH_2CH_2-$ unit at each turn of the cycle.[1] Reduction of the 3-ketoacyl product of the condensation is stereospecific and gives rise to the (3*R*)-hydroxyacyl intermediate[2,3] which in turn loses water in the dehydration to give exclusively the *trans*-2-enoyl derivative.[2] Previous investigations in this laboratory[4,5] have established the existence of an overall stereospecificity in the reactions catalysed by the fatty acid synthetase. A recent paper[6] has provided further evidence in support of the proposed concerted displacement mechanism[1,7] for the condensation reaction. Acceptance of this mechanism of condensation

implicates the dehydration reaction as the point at which the stereospecific elimination of a proton (originally present as one of the malonate methylene hydrogens in malonyl-CoA) must occur.[5] It is essential for the further evaluation of our earlier results[5] to know whether the *pro*-2*R* hydrogen (*anti*-elimination) or the *pro*-2*S* hydrogen (*syn*-elimination) is eventually removed with the (3*R*)-hydroxy-group during the dehydration.

Stereospecifically labelled (2*R*,3*R*)+(2*S*,3*S*)-3-hydroxy[2-$^3H_1$]butyric acid was synthesised by treating (*RS*)-2,3-epoxybutyric acid[8] with tritiated lithium borohydride,[9] a reaction that leads to a *trans*-opening of the epoxide ring.[10] The doubly racemic (2*RS*,3*RS*)-3-hydroxy[2-$^3H_1$]butyric acid was also prepared, essentially according to a published procedure.[11] Following the addition of (3*RS*)-3-hydroxy-[3-$^{14}$C]butyric acid, the two specimens were purified[12] prior to conversion *via* the mixed anhydride[13] into the enzymically active *N*-acetylcysteamine thioesters.[2,14] These compounds were then used as substrates for assay[14] of the dehydrase activity of the fatty acid synthetase purified from bakers yeast (*Saccharomyces cerevisiae*).[14] Earlier investigations have shown that only the D- or (3*R*)-(−)-hydroxyacyl intermediates are involved in fatty acid biosynthesis in yeast[2] and pigeon liver.[3] Thus, in the present studies, the presence of labelled L- or (3*S*)-(+)-hydroxybutyryl molecules in the substrate is of no consequence, as these are not metabolised by the purified synthetase used in this work.[2]

Following incubation with the synthetase, portions of water from the medium were flash distilled (20 °C; reduced pressure) and the tritium content of the distillate was determined. The residual material was extracted into chloroform and the crotonyl-*N*-acetyl-cysteamine product (**2**) was separated from unchanged 3-hydroxybutyryl-*N*-acetyl-cysteamine substrate (**1**) by column chromatography on silicic acid, followed by recrystallization to constant specific activity and isotope ratio with carrier material.




SCHEME

The conversion of the non-specifically tritiated (2*RS*,3*RS*)-3-hydroxy[3-$^{14}$C,2-$^3H_1$]butyryl substrate ($^3$H/$^{14}$C = 17·5) into the corresponding *trans*-2-enoyl product (crotonyl-*N*-acetylcysteamine) resulted, as expected, in the release of approximately one half (51%) of the tritium initially present in the transformed molecules. The isotope ratio was 9·03, a decrease of 48% relative to the ratio in the substrate. The stereospecifically labelled (2*R*,3*R*)+(2*S*,3*S*)-3-hydroxy[3-$^{14}$C,2-$^3H_1$]butyryl substrate ($^3$H/$^{14}$C = 5·16), on the other hand, lost only 6·5% of its tritium, the isotope ratio in the product being 4·76, a decrease of 7·8%. Bearing in mind that only the (3*R*) molecules were involved in this conversion, and that the tritium in this stereospecifically labelled substrate was in the *pro*-2*R* position, it follows that for the tritium to be retained in the product the dehydration must involve the removal of the *pro*-2*S* hydrogen with the (3*R*)-hydroxy-group, a *syn*-elimination (see Scheme). Assignment of this stereochemistry for dehydration, together with our previous findings that tritium is retained preferentially from (2*S*)-[2-$^3H_1$]malonyl substrate,[5] enables us to deduce that the condensation reaction in fatty acid biosynthesis proceeds with inversion of configuration at carbon-2 of malonate.

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