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STEREOCHEMISTRY OF β-HYDROXYDODECANOYL THIOESTER DEHYDRATION CATALYZED BY FATTY ACID SYNTHETASE FROM BREVIBACTERIUM AMMONIAGENES

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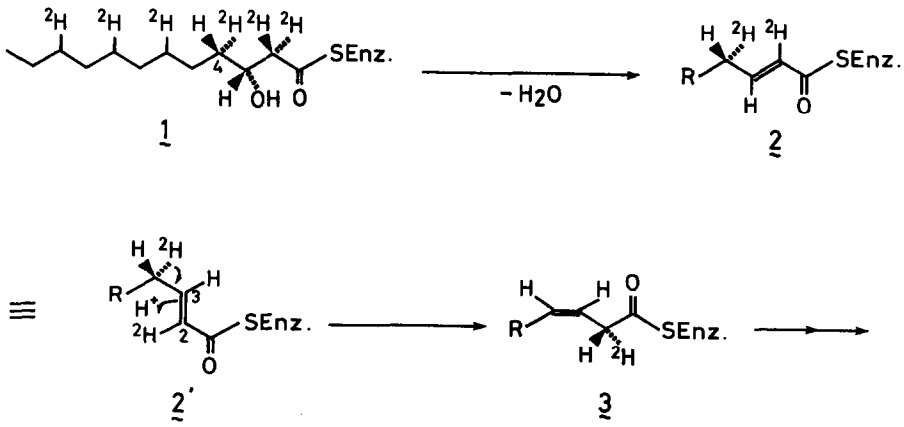
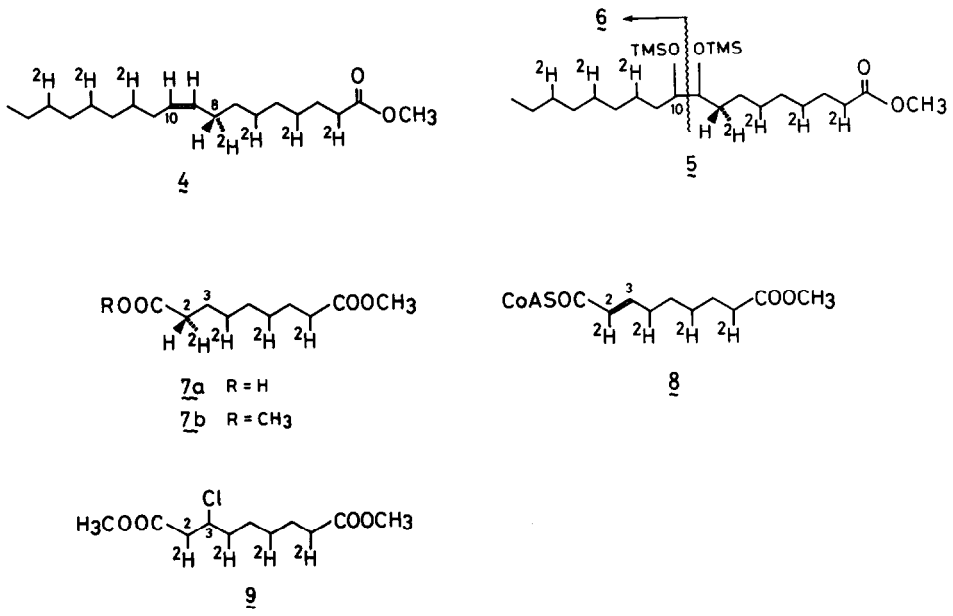
ABSTRACT: The pro-4S hydrogen of trans-α,β-dodecenoyl thioester was abstracted and a proton was added to the 2-Si face during the prototropic isomerization catalyzed by β-hydroxydodecenoyl thioester dehydrase.

The fatty acid synthetase complex from Brevibacterium ammoniagenes produces oleic acid by oxygen-independent pathway, which involves β,γ-dehydration of β-hydroxydodecanoyl thioester intermediate and subsequent chain elongation of β,γ-enoate without reduction of the double bond[2]. The enzyme component responsible for this dehydration is closely associated with the various activities for carbon chain elongation as an integral part of fatty acid synthetase complex. The reaction mechanism of the dehydration is similar to that catalyzed by -hydroxydecanoyl thioester dehydrase from Escherichia coli, which was elucidated by Bloch and coworkers[3]. They showed that trans-2-enoate 2 was first formed from β-hydroxy derivative 1 and cis-3-enoate 3 arose by prototropic isomerization of the conjugated enoate 2 . However, the stereochemistry of the isomerization has not been studied. In this communication we establish the stereochemical requirements of β-hydroxydodecanoyl thioester dehydration catalyzed by fatty acid synthetase from B. ammoniagenes.

Deuterium labeled oleic acid was synthesized from [2-$^2H_2$]malonyl-CoA by the synthetase and was methylated with HCl-CH_3OH[4]. Deuterium incorporation was not completely stoichiometric



Scheme I



Scheme II

because of the hydrogen exchange of [2-$^2H_2$]malonyl-CoA and the 'post malonate' exchange[5]. The methyl ester 4 was oxidized with OsO_4[6] and the resulting diol was converted to corresponding trimethylsilyl (TMS) ether 5 by N,O-bistrimethylsilylacetamide. TMS derivatives of unlabeled and biosynthesized oleic acid were applied to gas chromatography-mass spectrometry[7] and the mass spectra of the region corresponding to the ion of 6[8] are shown in Fig. 1. From these spectra it was demonstrated that three deuterium atoms were maximally present in the ion of 6. The synthetase produces palmitic and stearic acids in addition to oleic acid[2]. Deuterium distribution on saturated fatty acids synthesized from [2-$^2H_2$]malonyl-CoA was also analyzed and the results revealed that deuterium atoms located on every even-numbered carbon of saturated acids. Hence we deduced that no deuterium atom was distributed on C-10 of oleic acid which was synthesized by the enzyme from the accepted mechanism of fatty acid synthesis. As the deuterium atom derived from [2-$^2H_2$]malonyl-CoA was located on the pro-4S position of 1 by our previous studies[4], the pro-4S deuterium of 2' was abstracted during the prototropic isomerization.

The deuterium labeled methyl oleate 4 was cleaved by Lemieux-von Rudloff oxidation[9], and resulting monomethyl azelate 7a was subjected to the action of acyl-CoA synthetase and acyl-CoA oxidase which had been previously revealed to catalyze the anti elimination of pro-2R and pro-3R hydrogens of saturated acyl-CoA[10]. The resulting unsaturated acyl-CoA 8 was subjected to methanolysis and then converted to 3-chlorofatty acid methyl ester 9 by addition of hydrogen chloride[4]. The deuterium distribution of 7b was investigated by monitoring the ion of m/z 185 (M^+-OCH_3), m/z 186, m/z 187 and m/z 188 by 4 channels mass fragmentography[11]. The deuterium

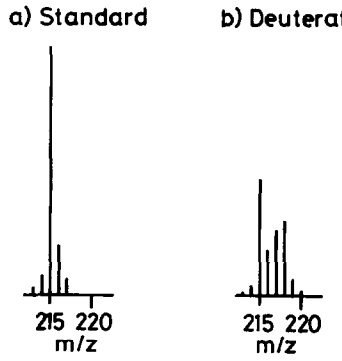


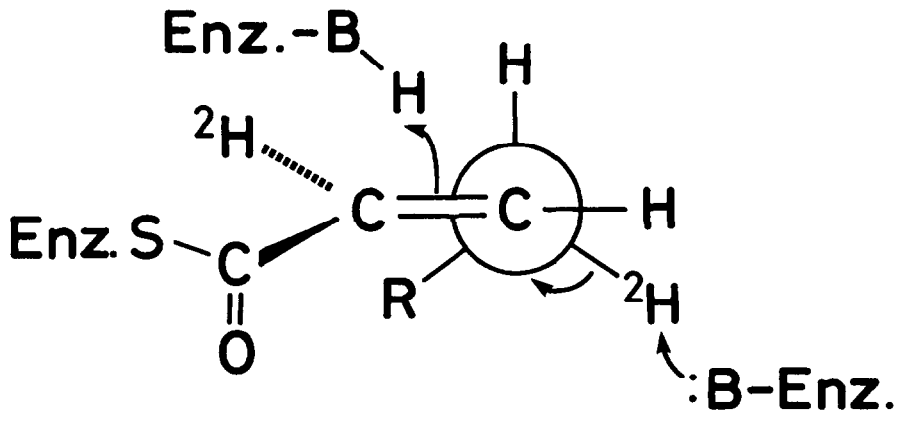
Fig. 1

Table 1. The observed and calculated intensities of the ion corresponding to M^+-(CH_3OH+Cl) region of 9 analyzed by 4 channels mass fragmentography[7].

m/z	Calculated intensities		Observed intensities
	assuming 2S of 7a	assuming 2R of 7a	
183	100	100	100
184	48.7	49.5	48.5
185	33.1	25.7	31.5
186	20.2	11.1	16.4

distribution of 7b was obtained by subtracting the experimentally observed natural isotopic abundance from the observed isotopic abundances of the biosynthetic samples as outlined by Biemann[12]. From this deuterium distribution we could estimate the relative intensities of each peaks in M^+-(CH_3OH+Cl) region of 9. In this case two possibilities exist, that is, the deuterium was incorporated into the pro-2S position of 7a and not abstracted by acyl-CoA oxidase, or the deuterium was incorporated into the pro-2R position of 7a and abstracted by acyl-CoA oxidase. The calculated and observed relative intensities of the ions corresponding to M^+-(CH_3OH+Cl) of 9 are shown in Table 1. These results suggested that the deuterium atom on C-2 of 7a was not abstracted by acyl-CoA oxidase and was incorporated into the pro-2S position of 7a[13]. Therefore the proton was added into 2-Si face of 2' during prototropic isomerization to form 3 .

We can summarize these results in Scheme III. The stereochemistry of the isomerization is consistent with a concerted mechanism of reaction whereby electrons from C-H bond of the pro-4S



Scheme III

hydrogen form the new double bond and release (from the opposite side of C-3) electrons for formation of the new C-H bond at C-2. And it is likely that acid-base catalysts on the enzyme assist in the manipulation of protons. These mechanism and stereochemistry are similar to those of isopentenyl pyrophosphate isomerase[14].

References and Notes

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