Stereochemical Course of the Hydration Reaction catalysed by β-Hydroxydecanoylthioester Dehydrase

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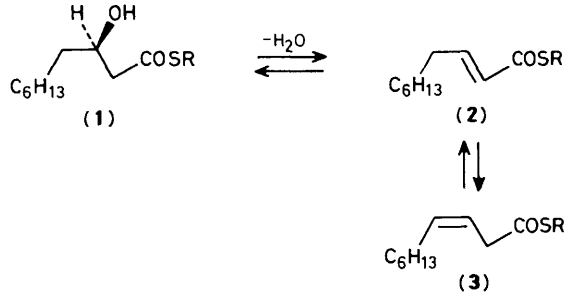
Enzyme-catalysed hydration of the *N*-acetylcysteamine thioester of (*E*)-dec-2-enoic acid to the corresponding thioester of (*R*)-3-hydroxydecanoic acid proceeds in a *syn* fashion, with protonation on the *si* face at substrate C-2.

β-Hydroxydecanoylthioester dehydrase,[1] the key enzyme in the biosynthesis of unsaturated fatty acids in bacteria under anaerobic conditions, mediates the interconversion of thioesters of (*R*)-3-hydroxydecanoic acid, (*E*)-dec-2-enoic acid, and (*Z*)-dec-3-enoic acid, (**1**), (**2**), and (**3**), respectively (R = acyl carrier protein, *in vivo*). We present evidence herein that the dehydrase-catalysed hydration of (**2**) to give (**1**) is a *syn* process, a result that is stereochemically and mechanistically consistent with previous findings[2—4] for the allylic isomerization of (**2**) to (**3**).

Figure 1(a) portrays the C-2 region of the 400 MHz ^{1}H n.m.r. spectrum of unlabelled 3-hydroxydecanoyl-NAC (NAC = *N*-acetylcysteamine). Clearly, this is the AB portion of an ABX system. Analysis shows that H_A and H_B resonate at δ 2.75 (J_{AX} 3.3 Hz) and 2.68 (J_{BX} 8.6 Hz), respectively, with J_{AB} 15.4 Hz. Assuming that the preferred conformation of 3-hydroxydecanoyl-NAC is governed by hydrogen bonding between the hydroxy group and the carbonyl oxygen (with the heptyl substituent in a quasi-equatorial orientation), absolute signal assignments can be made. Based on vicinal couplings,[5] the higher and lower field C-2 resonances represent H_S and H_R, respectively.

(*E*)-[2-^{2}H]Dec-2-enoyl-NAC[4] and unlabelled (*E*)-dec-2-enoyl-NAC were incubated with dehydrase in ^1H_2O and



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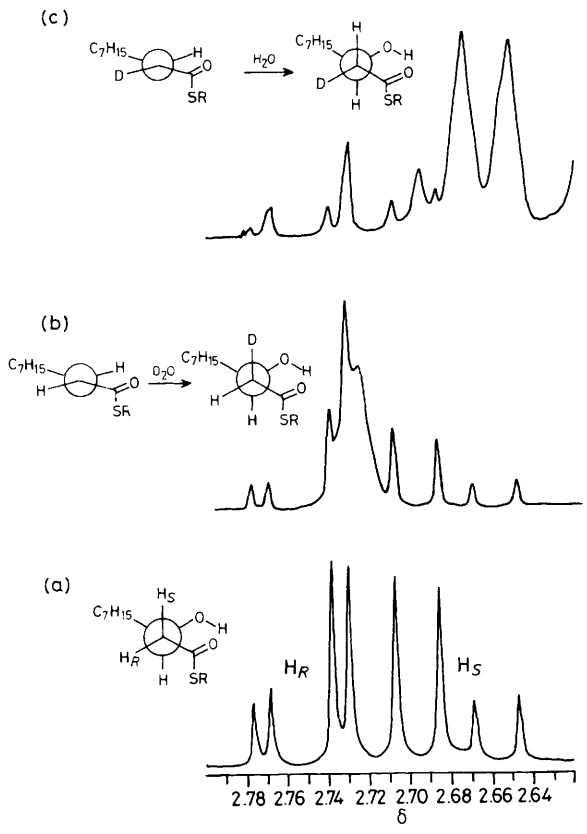

Figure 1. 400 MHz ^1H n.m.r. spectra of unlabelled and labelled 3-hydroxydecanoyl-NAC. The scale-expanded C-2 proton regions of (a) unlabelled (*RS*)-3-hydroxydecanoyl-NAC, (b) (2*S*,3*R*)-[2-$^2H_1$]-3-hydroxydecanoyl-NAC, from enzyme-catalysed hydration of unlabelled (*E*)-dec-2-enoyl-NAC in ^2H_2O, and (c) (2*R*,3*R*)-[2-$^2H_1$]-3-hydroxydecanoyl-NAC from enzyme-catalysed hydration of (*E*)-[2-^2H]dec-2-enoyl-NAC in ^1H_2O. The samples were dissolved in $CDCl_3$, and internal $SiMe_4$ was used as standard.

^2H_2O, respectively, as previously described.[4] (3*R*)-3-[2-$^2H_1$]Hydroxydecanoyl-NAC was isolated from each incubation mixture and the ^1H n.m.r. spectrum of each sample was obtained (Figure 1). Spectrum (b) is that of hydroxythioester from incubation of unlabelled substrate in ^2H_2O. A narrow doublet is evident at δ 2.73 (overlapping peaks stemming from unlabelled compound), corresponding to H_R. The spectrum of hydroxythioester from the complementary incubation, however, shows [Figure 1(c)] a broadened doublet (*J* 8.2 Hz) centred at δ 2.66 (in addition to resonances attributable to the presence of a small amount of the unlabelled compound). Clearly these results are complementary and indicate that in each case, protonation has occurred on the *si* face at C-2 of (*E*)-dec-2-enoyl-NAC. The specific coupling patterns observed are entirely consistent with expectation, as in each case the geminal H–D coupling is reduced to *ca.* 1/6.5 of the corresponding H–H value (15.4/6.5 = 2.4 Hz). The small discrepancies in the chemical shifts of the C-2 protons of the labelled *vs.* the unlabelled hydroxythioester are readily explained in terms of the well known isotope shift.[6]

Enzymes catalysing *syn*[7] and *anti*[8] hydration–dehydration reactions are well known. The present finding was predictable in that (a) the C-3-OH group of the hydroxythioester is in the *R* configuration, and (b) the dehydrase-catalysed isomerisation of (**2**) to (**3**) is known[3,4] to involve protonation of the former on the *si* face at C-2. The steric courses of the various dehydrase-catalysed reactions[2—4,9] are consistent with one another and strongly implicate[10] a *single* active site base, a histidine residue.[11] For stereoelectronic reasons it is clear that dehydrase-catalysed reactions involve delocalized carbanionic (or enolic) intermediates and are not concerted processes.

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