β-Hydroxydecanoylthioester Dehydrase. Steric Course at Substrate C–4 in the Enzyme–catalysed Allylic Rearrangement

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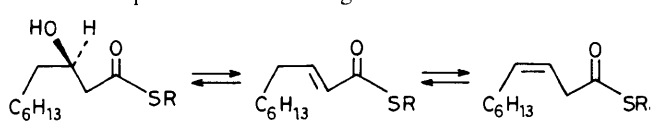
The *pro*-(4*R*) hydrogen is lost in the enzyme-catalysed rearrangement of the *N*-acetylcysteinamine thioester of *E*-dec-2-enoic acid to the corresponding thioester of *Z*-dec-3-enoic acid.

β-Hydroxydecanoylthioester dehydrase ('dehydrase'),[1] the pivotal enzyme in the biosynthesis of unsaturated fatty acids[2] by anaerobic micro-organisms, mediates the interconversion of acyl carrier protein (ACP) thioesters of (*R*)-3-hydroxydecanoic acid (**1**), *E*-dec-2-enoic acid (**2**), and *Z*-dec-3-enoic acid (**3**).

Extensive studies[1] on dehydrase by Bloch and his coworkers have resulted in both a clearer picture of structural and mechanistic imperatives for biochemical dehydrations as well as the pioneering demonstration[3] of 'suicide' enzyme inactivation.[4] Nevertheless, questions remain in regard to the number[5] and orientation of catalytically significant functional groups at the active site of dehydrase. We now report the results of experiments on the steric course of deprotonation at C-4 in the dehydrase-catalysed rearrangement of the *N*-acetylcysteinamine (NAC)† thioester of *E*-dec-2-enoic acid to that of *Z*-dec-3-enoic acid.

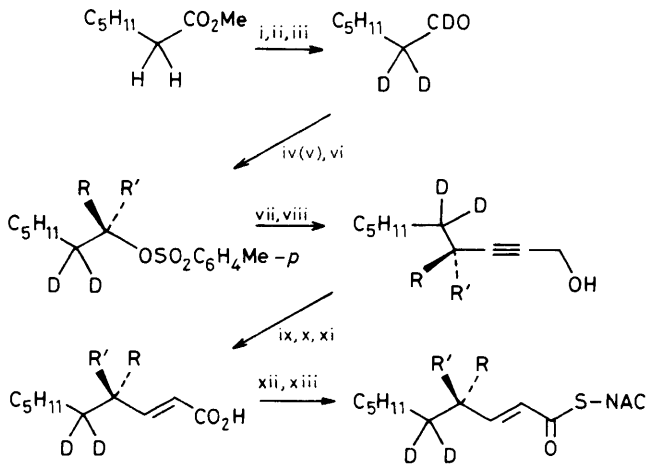
Syntheses of (4*R*)- and (4*S*)-*E*-[4,5,5-$^2H_3$]dec-2-enoyl NAC followed the route shown in Scheme 1. ^2H N.m.r. analysis of the chirally-labelled thioesters showed C-4 : C-5 deuterium ratios of 0.48 ± 0.01 and 0.51 ± 0.01, for the (4*R*) and (4*S*) forms, respectively. Furthermore, the ^1H n.m.r. spectra of the same samples showed vinyl proton coupling patterns and diminished C-4 proton signals which were consistent with the extents and positions of labelling as shown.



R = ACP or NAC

† Although ACP thioesters are utilized *in vivo*, nearly all previous investigations have been carried out with the more easily synthesized NAC derivatives.

Dehydrase was isolated rapidly and efficiently by the procedure of Cronan and Lakshman,[14] using *Escherichia coli*



(4*R*)-[4,5,5-$^2H_3$]-(**2**); R=H, R′=D

(4*S*)-[4,5,5-$^2H_3$]-(**2**); R=D, R′=H

Scheme 1. i, NaOMe, NaOD; ii, $LiAlD_4$, Et_2O; iii, CrO_3, pyridine; iv, 9-borabicyclo[3.3.1]nonane (9-BBN), (+)-α-pinene, (ref. 6) for R = H, R′ = D; v, 9-BBN, (−)-α-pinene, (ref. 6) for R = D, R′ = H; vi, *p*-$MeC_6H_4SO_2Cl$, pyridine; vii, $LiC{\equiv}CCH_2OTHP$ (THP = tetrahydropyran-2-yl), hexamethylphosphoramide (ref. 7); viii, *p*-$MeC_6H_4SO_2OH$, MeOH; ix, $LiAlH_4$, NaOMe,. tetrahydrofuran (THF) (ref. 8); x, MnO_2, pentane (ref. 9); xi, $NaClO_2$, Bu^tOH (ref. 10); xii, Ph_2POCl, CH_2Cl_2;¶ xiii, $Tl[CH_2]_2NHAc$, THF.¶

¶ This sequence, a variant on published procedures for thioesterification (see refs. 11, 12, and 13), gave consistently high yields. Details will be presented elsewhere.

DM51 A (a cloned, overproducing mutant strain‡) as the source of enzyme. Each labelled substrate sample was incubated with the partially purified enzyme preparation long enough to achieve equilibrium between (**1**), (**2**), and (**3**). At this time, the reaction was terminated by addition of Tris (to effect buffering at pH 8.5), THF, and solid $NaBH_4$. Under the conditions used, $NaBH_4$ quickly reduced the thioester groups, *E*-dec-2-enoate preferentially undergoing conjugate attack, leading ultimately to the isolation of relatively large amounts of decan-1-ol and decane-1,3-diol accompanied by smaller quantities of *E*-dec-2-enol and *Z*-dec-3-enol. Following preliminary column chromatography, the crude mixture was treated with MnO_2,[9] which oxidized the unwanted allylic alcohol to the corresponding aldehyde, and left the desired homoallylic alcohol unchanged. Finally, aroylation both increased the molecular weight (hence, the mass) of the product alcohol and provided a chromophore, by which the effluent from preparative h.p.l.c. could be monitored.

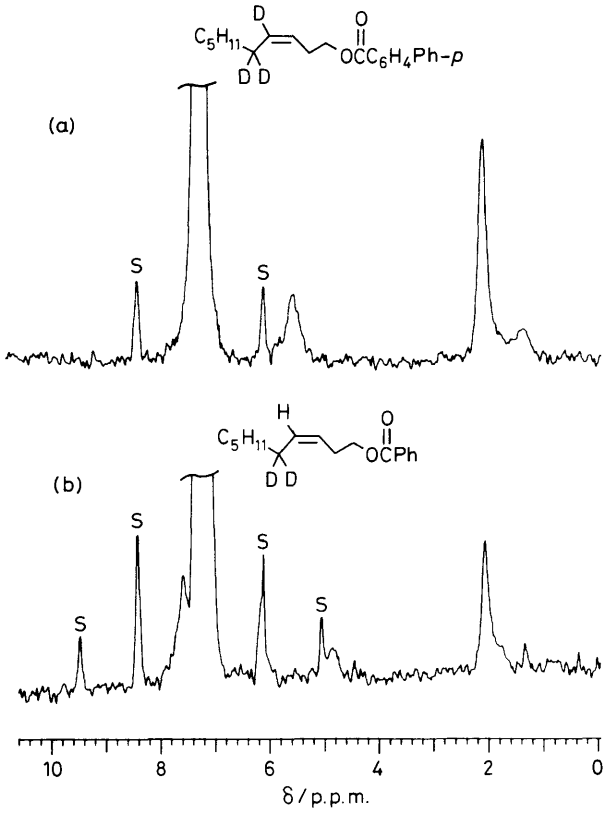


Figure 1. ^2H N.m.r. spectra of esters derived from incubation of (a) (4*S*)-[4,5,5-$^2H_3$]-(**2**) and (b) (4*R*)-[4,5,5-$^2H_3$]-(**2**) with dehydrase. The upper spectrum was run using external ^7Li lock, and the lower one with internal ^7Li lock. Each sample was dissolved in CCl_4, and $CDCl_3$ was used as the internal standard (δ 7.26 p.p.m.). Spinning sidebands are denoted by the symbol 'S'. In the lower spectrum, two extraneous signals are present, at δ 4.8 and 7.6 p.p.m. These result from the presence of impurities in the *Z*-dec-3-enyl benzoate sample and *not* from deuterium substitution in this allylic ester; the ^1H n.m.r. spectrum of unlabelled *Z*-dec-3-enyl benzoate shows no resonances at these chemical shifts.

‡ Dehydrase isolated from this strain is identical to that which is isolated from the wild type organism (J. E. Cronan, Jr., personal communication to J. M. S.).

^2H N.m.r. spectra of the homoallylic esters derived from incubations of the chirally-labelled substrates (**2**) with dehydrase are shown in Figure 1. The spectrum of *Z*-dec-3-enyl *p*-phenylbenzoate [Figure 1(a); from (4*S*)-(**2**)] comprises two resonances: at δ 5.57 (C-4) and 2.08 p.p.m. (C-5), in a ratio of 0.48 ± 0.01. After correction for the enantiomeric purity of the labelled substrate,§ the C-4 : C-5 ratios of the (4*S*)-substrate and the derived product are found to be within experimental error of each other. In Figure 1(b), the *only* peak assignable to *Z*-dec-3-enyl benzoate [from (4*R*)-(**2**)] is the C-5 signal at δ 2.08 p.p.m.

Clearly, it is the *pro*-(4*R*) hydrogen which is removed from (**2**) in the course of rearrangement to (**3**).

Recently, a Japanese group investigating the fatty acid synthetase of *Brevibacterium ammoniagenes* reported[15] removal of the *pro*-(4*S*) hydrogen in the course of an allylic rearrangement similar to that catalysed by dehydrase from *E. coli*. While there is no basis for *direct* comparison of these two enzymes (as they are from different organisms), it would appear that if the result of the Japanese researchers is correct, the enzymes are probably not related evolutionarily, only functionally.

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§ The optical purities of the samples of (−)- and (+)-α-pinene used were 89.3 and 98.4%, respectively. These figures are based on optical rotations at the sodium D line of −40.17 and +49.46° (neat), respectively.