β-Hydroxydecanoylthioester Dehydrase. Steric Course at Substrate C-2 and Overall Steric Course of the Enzyme–catalysed Allylic Rearrangement

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β-Hydroxydecanoylthioester dehydrase (*Escherichia coli*) catalyses the addition of a proton to the *si* face at C-2 of the *N*-acetylcysteinamine thioester of *E*-dec-2-enoic acid in isomerizing this substance to the corresponding *Z*-dec-3-enoic acid thioester, with the result that the overall steric course of the allylic rearrangement is suprafacial.

In the preceding paper[1] we defined the steric course of the deprotonation at C-4 in the allylic rearrangement of the *N*-acetyl-2-mercaptoethylamine (*N*-acetylcysteinamine; NAC) thioester of *E*-dec-2-enoic acid (**1**) to the NAC thioester of *Z*-dec-3-enoic acid (**2**), catalysed by the *Escherichia coli* enzyme β-hydroxydecanoylthioester dehydrase.[2] Herein we report the results of experiments which prove that protonation of (**1**) occurs from the *si* face at C-2 and that the overall steric course for the rearrangement is suprafacial.

E-[2-^{2}H]Dec-2-enoic acid was synthesized by procedures described in the preceding paper,[1] with introduction of label from lithium aluminium deuteride by reduction of dec-2-yn-1-ol in the presence of sodium methoxide.[3] The labelled acid was thioesterified[1] by conversion into the mixed anhydride (using diphenylphosphinyl chloride), followed by reaction with the thallium(I) salt of *N*-acetylcysteinamine.

Thioester (**1**) was incubated† with dehydrase until equilibrium had been achieved (45 min). At this point, solid ammonium sulphate was added until the reaction mixture had become saturated, and the thioesters were removed by repeated extraction with tetrahydrofuran. Following preliminary purification by flash chromatography,[4] the thioester mixture was subjected to preparative h.p.l.c., ultimately affording a small quantity of (**2**).

The configuration of the deuterium label at C-2 of (**2**) was determined by the method of Parker.[5] The applicability of this technique was proven by 76.88 MHz ^{2}H n.m.r. analysis of the methyl mandelate diesters of [2,2-$^2H_2$]-[6] and (*R*)-[2-$^2H_1$]-decanoic acid.[7] The spectrum of the former showed two signals, at δ 2.17 and 2.26 p.p.m. (relative to benzene, which was set to δ 7.15 p.p.m.), while that of the latter comprised a single peak, at δ 2.26 p.p.m. In all of Parker's examples,[5] the *pro*-(*R*) proton (or deuteron) had, similarly, been found to resonate in the more downfield position.

Accordingly, purified, enzymatically-labelled *Z*-dec-3-enoyl NAC (**2**) was treated with an excess of sodium borohydride in a buffered medium, and the resulting *Z*-dec-3-en-1-ol was reduced [$(Ph_3P)_3RhCl$–H_2–benzene][8] to the saturated alcohol, which was oxidized ($RuCl_3$–$NaIO_4$–MeCN–CCl_4–H_2O)[9] to [2-$^2H_1$]decanoic acid. This material was esterified to the methyl ester of (*S*)-2-hydroxy-2-phenylacetic acid, giving a diester whose 76.88 MHz ^{2}H n.m.r. spectrum [Figure 1(b)] displayed a single peak, at δ 2.27 p.p.m. Thus, *E*-[2-^{2}H]dec-2-enoyl NAC (**1**) is protonated from the *si* face of the double bond at C-2, giving rise to (*R*)-*Z*-[2-$^2H_1$]dec-3-enoyl NAC (**2**).

The complementary experiment was performed, by incubation of unlabelled *E*-dec-2-enoate (**1**) with dehydrase in deuterium oxide medium.‡ Analogous work-up and derivatization gave a mandelate diester whose 76.88 MHz ^{2}H n.m.r. spectrum [Figure 1(a)] showed two peaks, at δ 2.18 and 2.26 p.p.m., with a ratio of integrated areas of *ca.* 2:1. The presence of a significant amount of deuterium in the *pro*-(*R*) position is almost certainly attributable to epimerization of the extremely base-sensitive labelled thioester (**2**) during borohydride reduction. Again, enzyme-catalysed protonation of (**1**) had occurred from the *si* face at C-2.

As we had previously shown[1] that dehydrase removes the *pro*-(4*R*) hydrogen of (**1**) in the conversion of (**1**) into (**2**), it is now clear that *the dehydrase-catalysed allylic rearrangement is*

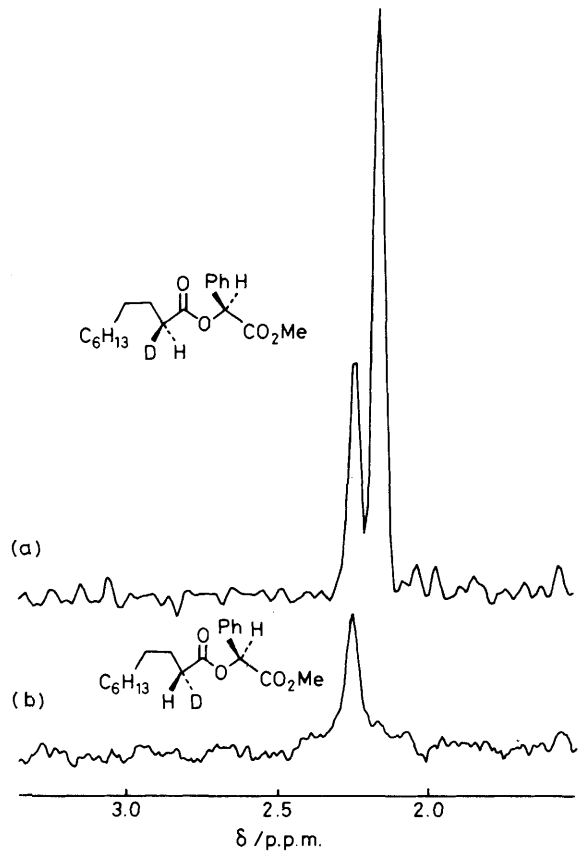
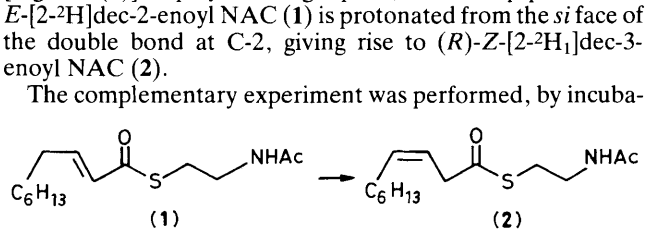
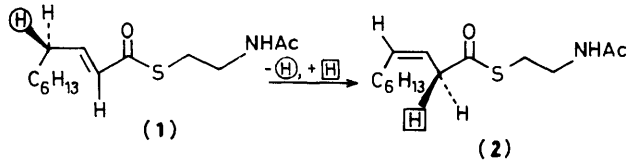


Figure 1. 76.88 MHz ^{2}H N.m.r. spectra of mandelate diesters derived from incubation of (a) unlabelled *E*-dec-2-enoyl NAC and (b) *E*-[2-^{2}H]dec-2-enoyl NAC with dehydrase in ^2H_2O and in ^1H_2O, respectively. The upper spectrum is resolution-enhanced. Protiobenzene was used as solvent, and the spectrometer was operated in the unlocked mode. Chemical shifts are referenced to the deuterium present at natural abundance in the solvent.



† *E*-Dec-2-enoyl NAC (200 mg) was incubated with 90 units of partially-purified dehydrase (see ref. 1). Reactions were conducted at 37 °C in 180 ml of 10 mM Tris–HCl, pH 7.0.

‡ This reaction was performed at an apparent pH of 6.6, owing to the non-equivalence of pH and pD (see ref. 10).



a suprafacial process. This result is suggestive of the involvement of a *single* base at the enzyme's active site.

The present result is consistent with the findings from studies of other enzymic allylic[11—13] and aza-allylic[14] rearrangements, most of which proceed *via* suprafacial proton transfer mechanisms. The exceptions include isopentenyl pyrophosphate isomerase,[15] and, as reported recently,[16] β-hydroxydecanoylthioester dehydrase of *Brevibacterium ammoniagenes*. The present result and its consistency with the majority of earlier findings suggests that a reinvestigation of the *Brevibacterium* enzyme may be in order.

Furthermore, the fact that a single base is most likely responsible for the *E. coli* dehydrase-mediated proton shift suggests that an earlier report[17] that the rearrangement is accompanied by *no* intramolecular proton transfer may be in error. It is mandatory that the latter experiment be repeated under single turnover conditions, using sensitive analytical methodology, enabling detection of even a small degree of intramolecular proton transfer.

We thank Professor J. E. Cronan, Jr., and Dr. M. Lakshman, both of the Department of Microbiology, University of Illinois, for making available to us *E. coli* DM51 A as well as a detailed procedure for purification of dehydrase. We also thank Ms. Barbara Berlett, N.I.H., for her co-operation and assistance in the use of the French pressure cell. High-field ^2H n.m.r. spectra were run by Dr. C. A. Evans, JEOL (U.S.A.), Inc., and by Dr. Luciano Mueller, at the Southern California Regional N.M.R. Facility at Cal Tech. Financial support was provided by the N.I.H.

Received, 13th October 1983; Com. 1357

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