133. Modelling the Metabolic Epimerization of Anti-inflammatory 2-Arylpropanoyl-coenzyme-A Conjugates: Solvent Effects on the $^1H/^2H$ Exchange in *S*-[2-(Dimethylamino)ethyl] 2-Phenylpropanethioate

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The $^1H/^2H$ exchange at the methine position of *S*-[2-(dimethylamino)ethyl] 2-phenylpropanethioate (DEPP) in solvent/D_2O mixtures was taken as a model reaction for the metabolic epimerization of 2-arylpropanoyl-coenzyme-A thioesters and was monitored by ^1H-NMR spectroscopy at 37°. The solvents used were (D_6)acetone, (D_3)acetonitrile, (D_6)dimethylsulfoxide, and (D_5)pyridine. In the investigated range of D_2O percentage (10–50%), the exchange reaction was found to increase linearily with D_2O content and with the basicity of the organic solvent, the fastest rates being close to 0.09 h^{-1} ($t_{½}$ *ca.* 8 h). These rates are slower than those observed *in vivo* for the configurational inversion of profens, and they are elicited in totally unphysiological concentrations of bases. The hypothesis thus formulated is that the metabolic epimerization of 2-arylpropanoyl-coenzyme-A thioesters cannot occur nonenzymatically.

Introduction. – Profens (2-arylpropionic acids) constitute a major group of anti-inflammatory drugs acting principally as inhibitors of prostaglandin synthetase [1]. Their *in vitro* activity resides almost exclusively in the (+)-(*S*)-enantiomers [2], yet all marketed profens except naproxen are racemates. These drugs also exhibit enantioselective pharmacokinetic behaviour, *e.g.* in serum albumin-binding [3][4] and in ester glucuronide formation [5–7].

The most intriguing aspect in the biotransformation of profens is their configurational inversion from the inactive (−)-(*R*)- to the active (+)-(*S*)-isomer, making the former a prodrug of the latter. The reaction has been documented *in vivo* for a number of profen analogues in a number of animal species, as comprehensively reviewed by *Hutt* and *Caldwell* [8], and as further proven by more recent evidence (*e.g.* [9–11]). The rate of configurational inversion appears to depend on both the animal species and the nature of the substrate, but no generalization can be offered at present [12]. In addition, a few recent findings suggest that the configurational inversion may not always be unidirectional [13].

Despite its clear pharmacodynamic consequences, the molecular mechanism of this configurational inversion has not been fully elucidated, presumably because the reaction has never been convincingly observed in subcellular preparations [14]. To date, there is overwhelming yet indirect evidence that conjugation with coenzyme A (CoA) to form 2-arylpropanoyl-CoA thioesters plays a central role in the reaction [8][13]. The configurational inversion is explained in terms of the acyl-CoA formation being substrate stereospecific for the (−)-(*R*)-enantiomer, while the (+)-(*S*)-enantiomer is not a substrate

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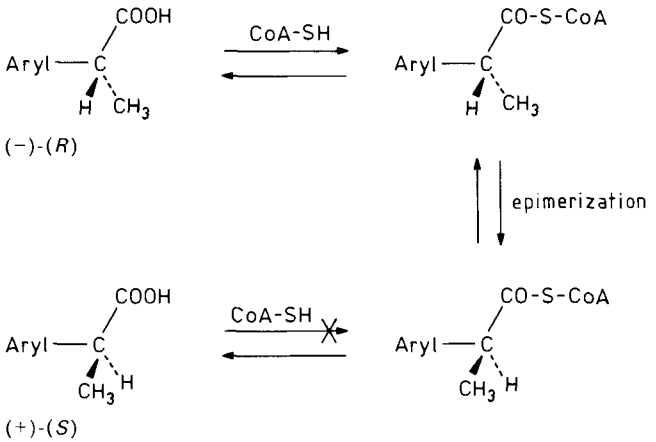
Scheme 1. *Postulated Mechanism for the Unidirectional Chiral Inversion of Profens* via *Epimerization of 2-Aryl-propanoyl-coenzyme-A Thioesters* [8]



for the acyl-CoA synthetase(s) catalyzing the reaction. The (*R*)-thioester may then epimerize to the (*S*)-thioester, since CoA is itself chiral, and the sequence is completed with the hydrolysis of the thioester (*Scheme 1*) [12].

Formation of CoA conjugates has been observed for a number of xenobiotic acids (*e.g.* [15]), but the mechanism of the epimerization step remains speculative. An enzymatic 2,3-dehydrogenation-reduction sequence was initially postulated but has been disproved by use of specifically deuterated substrates showing that inversion occurs with loss of the methine H-atom but without loss of a Me H-atom [16–18]. The configurational inversion at C(2) is, thus, commonly assumed to involve a simple (*i.e.* nonenzymatic) C(2)-deprotonation rendered possible by the strong acidity-enhancing effect of the thioester bridge [19].

To test this hypothesis, *S*-[2-(dimethylamino)ethyl] 2-phenylpropanethioate (DEP) was synthesized as a model of an acyl-CoA thioester, and the kinetic acidity of its CH proton was investigated by monitoring the rate of $^1H/^2H$ exchange occurring in deuterated solvents (*Scheme 2* and *Fig. 1*) [14]. Like CoA, 2-(dimethylamino)ethanethiol is a β-functionalized ethanethiol, having, however, a simple Me_2N group instead of an NH_2 group amidified by the bulky ATP-pantothenic acid. The rationale for the selection of DEPP was the presence of a polar β-substituent and the absence of the bulky ATP-pantothenoyl moiety.

It may be argued that the presence of a β-amino instead of a β-amido group can induce intramolecularly catalyzed hydrolysis of the thioester bridge. This catalysis, however, should not affect the relevance of our study for the following two reasons. First, all results of the present study were obtained with DEPP in the non-protonated state, and it was observed for 2-aminoethyl esters that intramolecularly catalyzed hydrolysis is *ca.* thousand times slower for the neutral than for the *N*-protonated form [20]. Second, and more important, the study aims at modelling the epimerization and not the hydrolysis of acyl-CoA thioesters, for which DEPP appears to us as a fully satisfactory model compound. What this study assumes, but does not investigate, is, whether rates of methine-

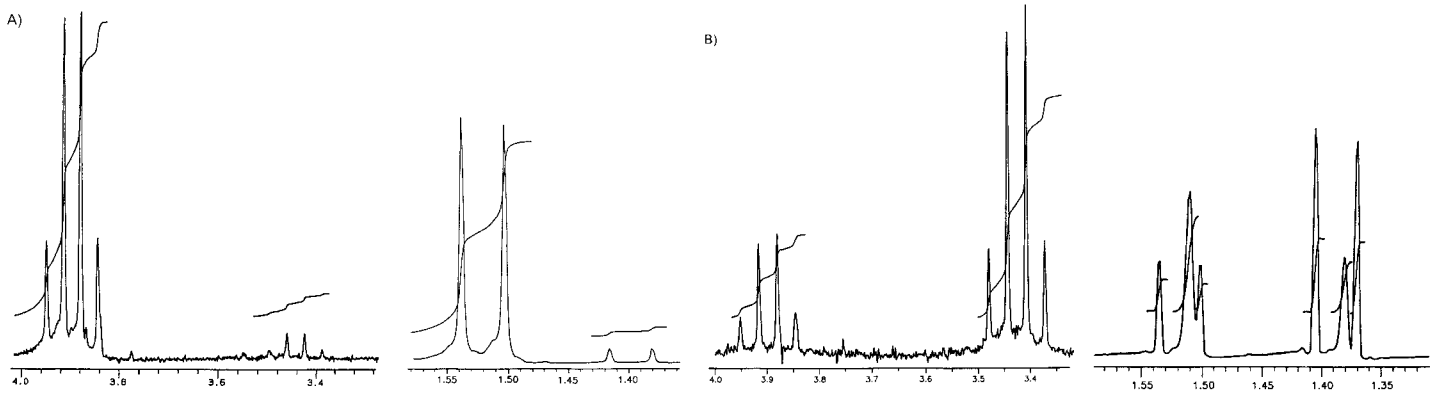


Fig. 1. *Partial 200-MHz ^1H-NMR spectra recorded during the $^1H/^2H$ exchange and hydrolysis of DEPP in CD_3CN/D_2O (50:50 w/w) at 37 . A:* Partial spectra recorded after 98 min. *Left panel* shows the *quadruplet* at 3.89 ppm (CH group in DEPP) and the *quadruplet* at 3.42 ppm (CH group in 2-phenylpropionate). On the *right panel* are displayed the *doublets* at 1.52 and 1.39 ppm (CH_3 groups in DEPP and 2-phenylpropionic acid). At this time, 5.4% $^1H/^2H$ exchange and 3.8% hydrolysis had occurred. *B:* Partial spectra recorded after 1347 min, where 41.2% $^1H/^2H$ exchange and 26.9% hydrolysis had taken place. *Left panel: quadruplets* at 3.89 and 3.42 ppm of the CH group in DEPP and 2-phenylpropionate, respectively. *Right panel: doublets* at 1.52 and 1.39 ppm of the CH_3 groups in DEPP and 2-phenylpropionic acid. Between the *doublets* are situated at 1.51 and 1.38 ppm the *singulets* of the CH_3 groups in mono-deuterated DEPP and 2-phenyl[2-$^2H_1$]propionic acid, respectively.

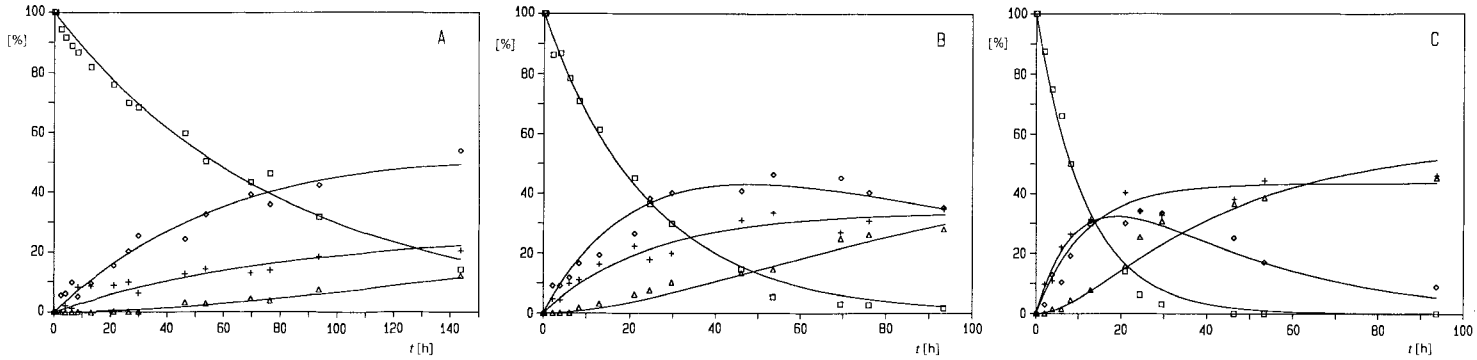
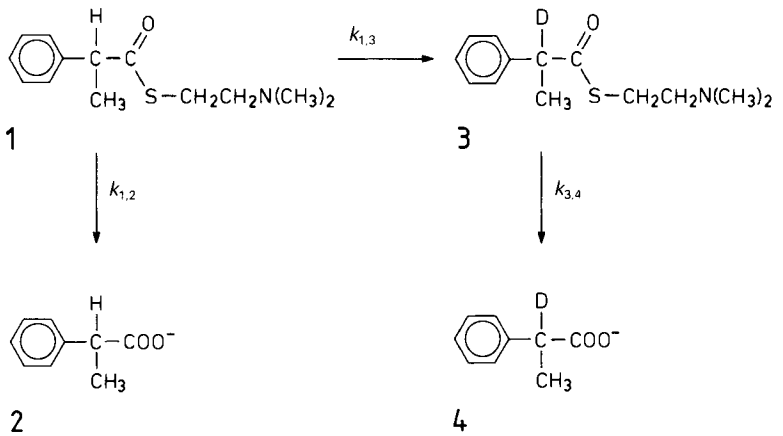


Fig. 2. *Time course of hydrolysis and $^1H/^2H$ exchange at the methine group of DEPP* (**1**) *in $(CD_3)_2CO/D_2O$ mixtures at 37°. A:* 90:10 *(w/w)*; *B:* 70:30 *(w/w)*; *C:* 50:50 *(w/w)*. The structure of **1–4** and the reaction scheme are given in *Scheme 2*. The points represent the experimental values (**1**: □; **2**: +; **3**: ◇; **4**: △), while the curves were calculated by nonlinear least-square analysis.

Scheme 2. *Chemical Reactions of Hydrolysis and $^1H/^2H$ Exchange Observed in the Investigated Deuterated Solvent/D_2O Mixtures.* The compounds are: *S*-[(dimethylamino)ethyl] 2-phenylpropanethioate (**1**); *S*-[2-(dimethylamino)ethyl] 2-phenyl[2-$^2H_1$]propanethioate (**3**); 2-phenylpropionic acid (**2**); 2-phenyl[2-$^2H_1$]propionic acid (**4**).



proton exchange indeed correlate with rates of racemization. This assumption, however, is reasonable, considering the similarities in reaction mechanisms [18].

A preliminary investigation showed that DEPP was inert up to 22 h in D_2O at 37° and pD 7.4, while in CD_3OD or CD_3OD/D_2O only solvolysis occurred. In contrast, competitive hydrolysis and $^1H/^2H$ exchange were observed in a CD_3CN/D_2O 50:50 mixture at 37°. This suggested that the kinetic acidity of the methine proton in 2-arylpropanoyl-CoA analogues must be markedly solvent-dependent, and that chemical epimerization of acyl-CoA thioesters could perhaps occur in a microenvironment of adequate polarity. To further examine this possibility, we have examined the kinetics of $^1H/^2H$ exchange of DEPP in four organic solvents containing fixed proportions of D_2O (10, 30 and 50% (*w/w*)). The organic solvents were chosen on the basis of their polar, water-soluble, and aprotic character. The results obtained do not support the conclusion that racemization can proceed nonenzymatically under biological conditions.

Results. – *Qualitative Examination of Experimental Results.* The time course of hydrolysis and $^1H/^2H$ exchange of DEPP in three different $(CD_3)_2CO/D_2O$ mixtures is shown in *Fig. 2*. Comparable patterns were seen in the CD_3CN/D_2O and $(CD_3)_2SO/D_2O$ mixtures. For these three organic solvents but not for (D_5)pyridine (see later), a number of qualitative observations can be made.

First, the rate of disappearance of DEPP increased with increasing D_2O content in the solvent mixture, and as expected the rate of hydrolysis of both thioesters (*i.e.* **1** and **3**) was proportional to the amount of heavy water present. The initial rate of formation of **3** was slow at low D_2O concentrations and became more rapid at higher D_2O concentrations. In contrast, the AUC (area under the curve) of deuterated thioester **3** decreases with increasing D_2O content.

Second, comparing the kinetics in $(CD_3)_2CO$, CD_3CN, and $(CD_3)_2SO$ with the same D_2O content reveals that the solvent properties greatly influenced the rate and extent of

DEPP disappearance, which was fastest in $(CD_3)_2SO$ and slowest in $(CD_3)_2CO$. Formation of the acid **2** increased in the order $(CD_3)_2SO < (CD_3)_2CO < CD_3CN$, while the formation of the deuterated thioester **3** decreased in that order. Hydrolysis of **3** again was faster in $(CD_3)_2SO$ than in $(CD_3)_2CO$ and CD_3CN.

In (D_5)pyridine/D_2O mixtures the fate of DEPP was different from that observed in the three other solvents. Indeed, the absence of a detectable second *quadruplet* and *doublet* in the NMR spectra indicated that no hydrolytic reaction had occurred. Only $^1H/^2H$ exchange was observed, its rate increasing with increasing D_2O concentrations.

Rate Constants of Hydrolysis and $^1H/^2H$ Exchange. The individual rate constants corresponding to *Scheme 2* were calculated using a kinetic model assuming unidirectional transformations, and the results obtained for the various solvent/D_2O mixtures are tabulated in the *Table*. For all four organic solvents, the three rate constants show a marked increase with increasing concentration of D_2O. However, the importance of this increase depends on both the organic solvent and the rate constant considered. For the rate constant of $^1H/^2H$ exchange ($k_{1,3}$), the increase is the strongest in (D_5)pyridine (14-fold), more modest for $(CD_3)_2CO$ (*ca.* 5-fold) and CD_3CN (*ca.* 4-fold), and smallest for $(CD_3)_2SO$ (2-fold; see also later *Fig. 3*). As far as hydrolysis is concerned, the increase is most pronounced for $(CD_3)CO$ and smallest for CD_3CN, $(CD_3)_2SO$ being intermediate. Interestingly, the ratio of the rate constants of hydrolysis ($k_{1,2}/k_{3,4}$) is larger than unity in all cases except one, indicating a distinct secondary isotope ^2H effect in the reaction of thioester hydrolysis ($k_H/k_D = 1.36 \pm 0.23$, $n = 9$).

Table. *Rate Constants of Hydrolysis and $^1H/^2H$ Exchange* (defined in *Scheme 2*) *as Determined by NMR at 37°*

Solvent	D_2O [%]	Molar fraction D_2O	$k_{i,j}(h^{-1} \times 10^3) \pm$ S.D.			$k_{1,2}/k_{3,4}$	$k_{1,3}/k_{1,2}$	$k_{1,3}/k_{3,4}$
			$k_{1,3}$	$k_{1,2}$	$k_{3,4}$			
$(CD_3)_2CO$	10	0.312	8.93±0.33	3.25±0.25	2.32±0.48	1.40	2.75	3.85
	30	0.636	26.73±1.03	13.63±7.13	8.93±0.64	1.53	1.96	2.99
	50	0.803	48.66±3.20	37.39±2.57	29.56±2.91	1.26	1.30	1.65
CD_3CN	10	0.241	8.18±0.37	10.18±0.35	6.82±0.85	1.49	0.804	1.20
	30	0.554	18.31±0.76	21.40±0.71	14.76±1.60	1.45	0.855	1.24
	50	0.744	31.92±1.50	38.55±1.43	26.59±2.76	1.45	0.828	1.20
$(CD_3)_2SO$	10	0.304	44.28±1.59	2.64±0.72	1.62±0.45	1.63	16.8	27.4
	30	0.628	65.98±3.78	13.73±1.81	15.66±1.16	0.876	4.81	4.21
	50	0.797	89.69±5.29	23.95±2.57	20.48±1.40	1.17	3.75	4.38
(D_5)Pyridine	10	0.332	5.86±0.20	0.00	0.00			
	30	0.657	37.32±2.13	0.00	0.00			
	50	0.817	85.45±10.15	0.00	0.00			

The ratios $k_{1,3}/k_{1,2}$ for the two competitive reactions in *Scheme 2* indicate that the preference for $^1H/^2H$ exchange over hydrolysis is the largest in $(CD_3)_2SO$ (excepting (D_5)pyridine where it is apparently total) and smallest in $(CD_3)_2CO$; the reverse preference holds in CD_3CN (where it is independent of the D_2O content).

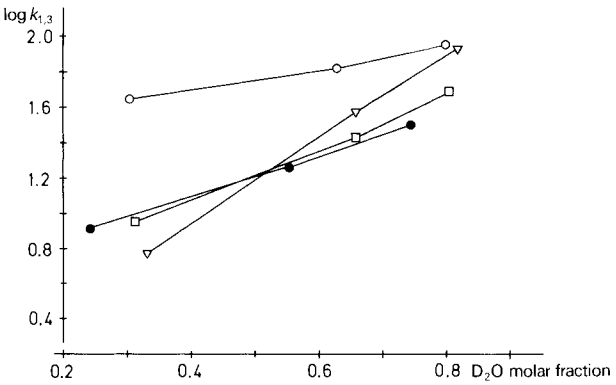


Fig. 3. *Plot of the logarithm of $^1H/^2H$ exchange rate constant* (log $k_{1,3}$) vs. *the molar fraction of D_2O present in the solvent mixture* (CD_3CN: ●; $(CD_3)_2CO$: □; $(CD_3)_2SO$: ○; (D_5)pyridine: ▽)

Factors Influencing the Rate Constant of $^1H/^2H$ Exchange. The aim of this work was to investigate the kinetic acidity of the methine proton and the factors influencing it, but the *Table* and the above presentation of data are not conducive to useful generalizations. As much as possible, quantitative relationships are more helpful. The observation that $k_{1,3}$ increases with the D_2O content of the solution leads to a quantitative examination of this relationship. As shown in *Fig. 3*, excellent linearity ($r^2 > 0.998$) is observed for all solvents between log $k_{1,3}$ and the molar fraction (MF) of D_2O. The individual regression equations are:

$$(CD_3)_2CO: \quad \log k_{1,3} = 1.50\ MF + 0.482 \tag{1}$$

$$CD_3CN: \quad \log k_{1,3} = 1.17\ MF - 0.626 \tag{2}$$

$$(CD_3)_2SO: \quad \log k_{1,3} = 1.45\ MF + 0.611 \tag{3}$$

$$(D_5)\text{pyridine}: \quad \log k_{1,3} = 2.41\ MF - 0.027 \tag{4}$$

Interestingly the slope in *Eqn. 4* is significantly different from that in *Eqns. 1–3*. Combining all the four organic solvents yields *Eqn. 5* (95% confidence limits in parentheses):

$$\log k_{1,3} = 1.45\ (\pm 0.81)\ MF + 0.627\ (\pm 0.493) \qquad n = 12;\ r^2 = 0.612 \tag{5}$$

Eqn. 5 shows that the D_2O concentration alone explains only 60% of the variance and is, thus, largely insufficient to account for the variation of the exchange rate constant. Reasoning that the basicity of the organic solvents or their capacity to interact with D_2O might influence the rate of exchange, we examined various physicochemical parameters encoding such properties, in particular dipole moment, boiling point, an index of solvent dipolarity/polarizability, and various cohesion parameters (dispersion, polar, and H-bonding). The only statistically useful descriptor was found to be the β parameter, *i.e.* a measure of the solvent's ability to act as H-bond acceptor [21]. The β values are: CD_3CN 0.31, $(CD_3)_2CO$ 0.48, (D_5) pyridine 0.64, and $(CD_3)_2SO$ 0.76. Introduction of this term into *Eqn. 5* yields *Eqn. 6:*

$$\log k_{1,3} = 1.36(\pm 0.65)\,\mathrm{MF} + 0.957(\pm 0.795)\,\beta + 0.157(\pm 0.553) \quad (6)$$

$$n = 12,\ r^2 = 0.787,\ F = 16.6,\ s = 0.20$$

This equation is statistically improved over *Eqn. 5* and confirms that the solvent's basicity is indeed an important factor influencing the rate of exchange. However, more than 20% of the variance remain unaccounted for, mainly due to the fact that *Eqn. 6* combines *Eqns. 1–3* of comparable slope with one *(Eqn. 4)* whose slope is different. Thus, additional solvent factors besides D_2O content and basicity must influence the rate of exchange, as suggested by the peculiar variation of the latter in (D_5)pyridine/D_2O mixtures. The nature of these factors could not be unravelled from the present data.

Discussion. – In this study, the $^1H/^2H$ exchange was taken as a model reaction for acyl-CoA-thioester epimerization, and solvent conditions were sought that would maximally increase the rate of exchange relative to the rate of hydrolysis. This was partly achieved in $(CD_3)_2SO$, and completely in (D_5)pyridine where no hydrolysis was detected even in the presence of 50% D_2O.

Such results would seem to suggest that conditions can exist in biological systems under which chemical (*i.e.* nonenzymatic) epimerization can occur without significant competition from chemical hydrolysis. However, a closer examination of the data reveals that such a conclusion is not warranted. Indeed, the largest $k_{1,3}$ values observed in the present study are close to 0.09 h^{-1}, corresponding to a $t_{1/2}$ of *ca.* 8 h. Such a half-life is too long to be compatible with the fast rate of configurational inversion observed *in vivo* for a number of profens. Furthermore, we note that the solvent systems, which lead to the $k_{1,3}$ values of *ca.* 0.09 h^{-1} ($(CD_3)_2SO/D_2O$ and (D_5)pyridine/D_2O 50:50), are totally unphysiological in their concentration of base. Increasing the percentage of D_2O beyond 50% might be expected to increase the rate of exchange up to a limit, but without rendering the medium more physiological.

For the reaction to proceed rapidly in biological systems (*i.e.* with $t_{1/2}$ in the order of 1 h, a microenvironment of particular basicity and/or reactivity is clearly indicated. Such conditions do not exist in biological fluids, but only in the active sites of some enzymes. We, thus, interpret our results to mean that the reaction of epimerization of 2-arylpropanoyl-CoA thioesters can only be enzyme-mediated. Confirmation of this hypothesis must await the characterization of the operative enzyme.

Experimental. – *Chemicals. S*-[2-(Dimethylamino)ethyl] 2-phenylpropanethioate (DEPP) was synthesized and analyzed as described in [14]. The deuterated solvents were obtained from commercial sources; their isotopic purities were $D_2O > 99.7\%$, $CD_3CN > 99.8\%$, $(CD_3)_2CO > 99\%$, $(CD_3)_2SO > 99.8\%$, and (D_5)pyridine $> 99.5\%$.

Kinetic Studies. The twelve media investigated consisted of mixtures of D_2O with CD_3CN, $(CD_3)_2CO$, $(CD_3)_2SO$, or (D_5)pyridine, the proportions in each case being 50:50, 70:30, and 90:10 *(w/w)*. DEPP (20 mg) as the free base was dissolved in 400 mg of solvent mixture. Each such soln. was placed in an NMR tube, kept in a water bath at 37°; at various time intervals up to six days, the ^1H-NMR spectra and integrals were recorded at 37° using a *Varian VXR-200* NMR spectrometer operating at 200 MHz.

Analysis. As previously shown [14], DEPP (**1**) in solvent/D_2O mixtures undergoes the reactions depicted in *Scheme 2, i.e.* thioester hydrolysis to produce 2-phenylpropionic acid (**2**) and $^1H/^2H$ exchange at the methine group to yield the deuterated thioester **3**. The latter can subsequently be hydrolyzed yielding 2-phenyl[2-$^2H_1$]propionic acid (**4**).

DEPP hydrolysis can be monitored by the following changes in the NMR spectra *(Fig. 1) a)* progressive replacement of the *quadruplet* around 3.9 ppm with a *quadruplet* around 3.4 ppm, and *b)* progressive replacement of the *doublet* around 1.5 ppm with a *doublet* around 1.35 ppm. $^1H/^2H$ Exchange in **1** to form **3** can be monitored by

the progressive replacement of the CH_3 *doublet* (around 1.5 ppm) with a *singulet*. Hydrolysis of **3** can be followed by integrating the *singulet* appearing at higher fields and corresponding to the CH_3 group in **4**. Thus, the kinetics of all four compounds in *Scheme 2* can be followed by integrating their characteristic signals. For the calculation of kinetic parameters, the four integrals were expressed as percentage of the total.

Kinetic Model. Preliminary calculations showed that the best fit is obtained assuming all transformations to be unidirectional, in accordance with previous observations [14]. Based on the kinetic model shown in *Scheme 2*, the following rate equations were employed for the determination of the rate constants:

$$\frac{d[\mathbf{1}]}{dt} = -(k_{1,3} + k_{1,2}) \cdot [\mathbf{1}]; \quad \frac{d[\mathbf{2}]}{dt} = k_{1,2} \cdot [\mathbf{1}]; \quad \frac{d[\mathbf{3}]}{dt} = k_{1,3} \cdot [\mathbf{1}] - k_{3,4} \cdot [\mathbf{3}]; \quad \frac{d[\mathbf{4}]}{dt} = k_{3,4} \cdot [\mathbf{3}]$$

where [**1**]–[**4**] represent the concentrations of compounds **1** to **4**. The calculations were performed by nonlinear least-square analysis.

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