

The primary kinetic hydrogen isotope effect in the deprotonation of a nitroalkane by an intramolecular carboxylate group

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The rates of racemization of optically active nitropentanoic acid, and 4-deuteronitropentanoic have been compared. The rate ratio (kie) is $k^H/k^D = 5.68(\pm 0.17)$ at 31°C, in good agreement with that determined by Lewis *et al.* for base-catalysed deprotonations using iodine-trapping methods. In a more detailed study, optically active 4-nitro-4-phenylbutanoic acid (NPBA) has also been prepared and rates of racemization measured in dimethoxyethane:water. With less than a full equivalent of triethylamine, rates are proportional to $[\text{Et}_3\text{N}]/[\text{NPBA}]$. For $1 < [\text{Et}_3\text{N}]/[\text{NPBA}] < 2$, rates are independent of the ratio, consistent with racemization being dominated by deprotonation of the nitroalkane by the intramolecular carboxylate group. The solvent isotope effect is $k^{\text{H}_2\text{O}}/k^{\text{D}_2\text{O}} = 0.73(\pm 0.04)$ and rates of exchange with D_2O are equal to rates of racemization. Comparison with rates of racemization by acetate of the methyl ester yielded an effective molarity (EM = 13.7) for the intramolecular carboxylate. The kie for racemizations of NPBA and 4-deutero-NPBA is $k^H/k^D = 5.78$ at 25°C, and for $20 < T < 50^\circ\text{C}$, $E_a^D - E_a^H = 5.5(\pm 0.1)$ and $A^H/A^D = 0.63(\pm 1.03)$. For the acetate catalysed racemizations of the methyl ester, 25°C, $k^H/k^D = 7.43$ with $E_a^D - E_a^H = 5.2 \text{ kJ mol}^{-1}$ and $A^H/A^D = 1.08$. In neither case is there any indication of a major tunnelling contribution on the isotopic rate ratio. A hitherto unrecognised mode of decomposition of nitronic acids, involving direct reaction with dissolved oxygen, has been identified. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: primary kinetic hydrogen isotope effect; racemizations; nitroalkane; intramolecular deprotonation; temperature dependences; effective molarity

INTRODUCTION

With the availability of three isotopes, and large isotopic mass ratios, kinetic hydrogen isotope effects (kies, k^H/k^D , k^H/k^T and k^D/k^T), are especially valuable in the characterisation of mechanisms of deprotonation of carbon acids, firstly to determine if a proton transfer is rate-limiting in a complex sequence and, when such a situation has been discovered, then to probe the nature of the proton transfer transition state. The usefulness of the method follows from an understanding of the origins of the kinetic effects at the molecular level. An excellent qualitative understanding has been available for many years, with kinetic isotope effects associated with changes in force constant of the vibrations of isotopically substituted bonds as reactants proceed from ground to transition state.^[1–4] Vibrational analysis, coupled with bond-energy bond-order relationships, has provided a useful empirical treatment to relate the experimentally determined isotope effects to transition state models.^[5] If, however, isotope effects are to be linked in a detailed way to changes in molecular structure, *ab initio* methods must be applied and high-level computational studies have been undertaken in a few cases. Since rate ratios for H- and D-isotopomers at 25°C are usually less than 8, quantitatively useful models must be capable of calculating activation energy differences much smaller than 5.7 kJ mol^{-1} presenting a tough challenge, even to modern computational methods.

The challenge to theory has become more pronounced with the accumulation of reports of enzyme-catalysed processes showing kinetic isotope effects considerably larger than those

anticipated from complete loss of ground state zero-point energy differentials.^[6–8] The temperature dependences of such rate ratios often yield Arrhenius parameters which are incompatible with explanations involving only ground state zero-point energy differences (for $20 < T < 2000 \text{ K}$, $E_a^D - E_a^H \approx 5 \text{ kJ mol}^{-1}$ and $0.7 < A^H/A^D < 1.32$).^[9] These effects (which are rare in 'normal' organic reactions) have been taken as evidence for mechanisms involving quantum mechanical tunnelling of the transferring proton through the barrier (from the Born–Oppenheimer potential energy surface).^[10,11] An emerging explanation^[12,13] of the occurrence of such contributions in enzyme-catalysed reactions is 'environmentally coupled or vibrationally assisted hydrogen tunnelling', focusing on the role of protein dynamics and qualitatively understood in terms of a strong coupling between certain vibrations of the reacting protein acting to narrow the barrier.^[14] Our own computational studies on methylamine dehydrogenase (MADH)^[15,16] showed that the structural configuration of an enzyme during transfer of a proton from the carbon of a Schiff base to the carboxylate of an aspartate (Fig. 1) can strongly affect the shape of the barrier to proton transfer and that large kies might then arise, in line with experiment. The dominating effect in these studies has been the

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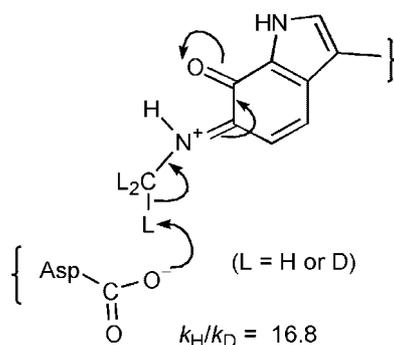


Figure 1. Carbon deprotonation in the active site of MADH

exo- or endothermicity of the process, which indirectly affects the shape of the barrier. In their current state, these studies rationalise rather than predict the results of experiment, and because of the size and complexity of proteins, difficulties arise in separation of the interacting contributions and inclusion of dynamical effects to experimental accuracy.

The theory thus remains unclear in its application in enzymes, and it has been pointed out^[17] that, while the chemistry occurring in the active site of enzyme-substrate complexes has often been modelled by intramolecular reactions in small molecules,^[18] measurements of kinetic isotope effects in intramolecular proton, hydride and hydrogen atom transfers in such models are rare. Indeed, a search for such measurements in intramolecular deprotonations of carbon acids revealed only the three examples shown in Fig. 2. All reactions were carried in aqueous medium, and in no case were temperature dependences reported.

Acetophenones **1** and **2** are from the work of Bell.^[19,20] In these, the acidic carbon site is adjacent to the ketonic carbonyl and phenolic or carboxylic acid groups provide the catalysing intramolecular base. In both cases, rates of enolization were determined by halogen trapping. An elevated isotope effect was reported for **1** but close reading of the original paper suggests that this result should be viewed with caution. Additionally, the acidic methyl groups were fully labelled, so observed ratios were combinations of primary and secondary effects which were not disentangled. The large difference between the acidities of the

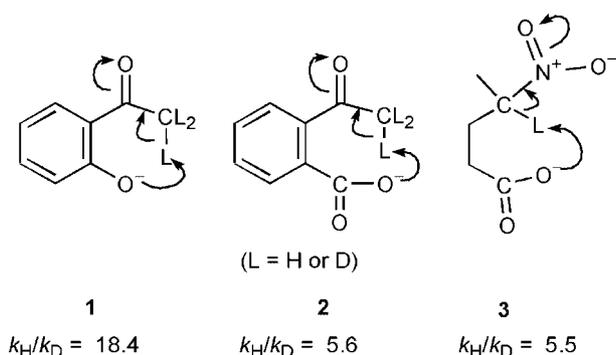


Figure 2. Available kinetic isotope effects in intramolecular deprotonations of carbon acids

catalysing groups (pK_a ca 10 for the phenol and ca 5 for the carboxylic acid) and that of the carbon acid (ca 20) shows that intramolecular carbon deprotonations would be strongly endothermic in both cases, a situation believed to disfavour tunnelling. The final example, in which there can be no complicating secondary effect, is 4-nitropentanoic acid, **3**, from the work of Lewis.^[21,22] In this case, the carbanion is stabilised by a nitro group, the best carbanion stabilising group in organic chemistry, with the anion best depicted as a nitronate with the negative charge largely on the oxygen atoms of the group. A $pK_a = 7.7$ is expected^[23,24] for the carbon acid, so that the imbalance with the catalysing carboxylic acid group is much reduced ($\Delta pK_a \approx 3$) compared to that in **1** or **2**. This, combined with the high kinetic barriers associated with deprotonation of nitroalkanes^[25–27] makes **3** the most promising of this group for tunnelling. In the event, the reported kie ($k^H/k^D = 5.5$ at 25°C), again from halogen trapping experiments, is rather less than those reported for intermolecular deprotonations of 2-nitropropane by other oxyanionic bases.^[28] No temperature dependence was reported.

In view of the sparsity of such data, we have begun a study of isotope effects associated with intramolecular proton transfers in synthetically accessible carbon acids, with the aim of providing measurements for simple reactions in which ground and transition states have been characterised as fully as experiment allows. Experimental and computational studies are to be compared, and results used to validate the theoretical models, leading eventually to a computational model with predictive power.

As noted earlier, values of k^H/k^D significantly larger than those which might be expected from complete loss of isotopically induced ground state zero-point energy differentials are rare in 'small molecule' chemistry, occurring mainly (but not exclusively) in deprotonations of nitroalkanes. Some of the very largest of these^[29–31] ($k^H/k^D = 50$ at 25°C) have, on reinvestigation, turned out to be artefacts arising from unrecognised loss of isotopic label during the rate measurements,^[32,33] but values between $14 < k^H/k^D < 20$ remain to be explained. These are often associated with deprotonations in aprotic media by hindered bases^[34,35] but deprotonations of 2-nitropropane by hindered pyridine bases in protic medium are particularly well characterised,^[36,37] with $k^H/k^D = 19.5$ at 25°C in EtOH, and temperature dependence (a 12°C range only!) giving $A^H/A^D = 7.1$ and $E_a^D - E_a^H = 12.5$ kJ mol⁻¹, compatible with the intervention of tunnelling. Pressure dependences also are compatible with a large tunnelling contribution in the ¹H-isotopomer,^[38] but the tritium isotope effect, k^H/k^T , has also been measured^[39] and the value of the exponent $\alpha = 1.42$ in the Swain–Schaad relationship,^[40] $k^H/k^T = (k^H/k^D)^\alpha$, does not support a high-tunnelling contribution.

It is perhaps worth repeating that the large kinetic isotope effects and unusual Arrhenius behaviour taken as indicative of proton tunnelling may also arise from mechanistic complexities. Quite reasonable combinations of microscopic rate constants and conventional primary isotope effects may produce such results,^[41,42] for example, in reactions in which products are formed from common intermediates. Even where kinetic isotope effects fall within the 'normal' range, anomalous Arrhenius behaviour may arise from a mechanism involving internal return.^[43] Checking for such complexities in enzyme-catalysed reactions is far from trivial but an essential part of any study of small molecule reactions intended to provide data for the construction and optimisation of theoretical models.

RESULTS AND DISCUSSION

Choice of substrate and experimental design

Nitroalkanes offer a combination of particularly high carbanion stability with high kinetic barrier to deprotonation (the nitroalkane anomaly^[25–27]), a combination thought to favour tunnelling pathways, and numerous detailed studies of intermolecular deprotonation exist for comparison. With the exception of Lewis' study of 4-nitropentanoic acid **3**, intramolecular variants have not been examined, but an oxidative conversion of nitroalkanes to corresponding aldehydes or ketones catalysed by the flavoenzyme, nitroalkane oxidase, has been shown to involve deprotonation of an enzyme-bound nitroalkane by the carboxylate group of an aspartate residue,^[44] with a remarkable 10^9 -fold rate enhancement over the acetate induced reaction. A kinetic isotope effect, $k^H/k^D = 7.9$, has been reported for the reaction of 1,1-dideuteronitroethane.^[45,46]

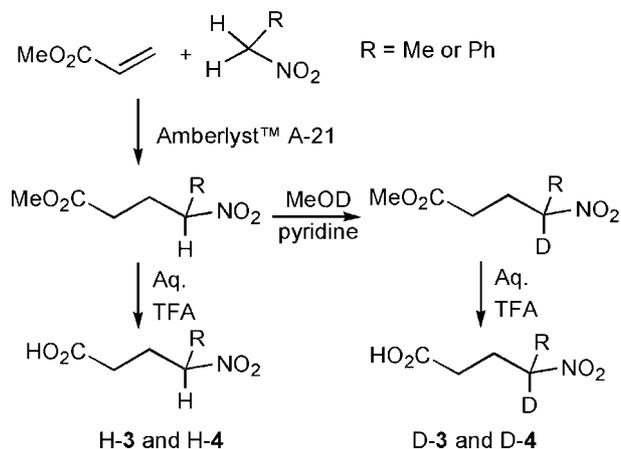
In this work, we have firstly re-examined the behaviour of **3** to establish techniques and methods, and then undertaken a more detailed examination of the behaviour of 4-nitro-4-phenylbutanoic acid **4** in which the methyl group of **3**, is replaced by phenyl. In both nitroalkanes, formation a nitronate anion eliminates the stereogenic centre adjacent to the nitro group, so that loss of optical activity from resolved compounds offers a useful handle on extent of the deprotonation reaction. Replacement of methyl by phenyl induces a favourable small shift of the carbon acidity (7.39^[47] as opposed to 7.7^[23,24]) and offers the valuable technical advantages of enhanced UV activity and ease of preparation of optically active material, but is not expected to make major changes to the chemistry.

Preparative considerations and preliminary reactivity studies.

Methyl esters of both **3** and **4** are readily accessible by conjugated addition of nitroethane or phenyl nitromethane to methyl acrylate,^[48] and pyridine-catalysed exchange with OD-methanol affords the required deuteriated materials. Extents of deuteration could be readily determined from ¹H-NMR spectra by integration since the signal from the exchangeable hydrogen adjacent the nitro group was well resolved (a doublet of doublets at δ 4.68 in **3** and δ 5.60 in **4**) and readily compared with signals from non-exchanging sites. As anticipated from the established behaviour of nitroalkanes,^[49,50] hydrolysis of the esters by heating in aqueous TFA occurred without exchange of hydron at the position α -to the nitro group.

In the case of **3**, we could not find conditions for resolution of either the ester or the acid by chiral chromatography, and resorted to an established method^[51] involving crystallisations of the quinine salt of the **3**, in both isotopomeric forms. Again, extents of deuteration after resolution were readily measured from ¹H-NMR spectra by integration, but, in our hands, the resolution by this method was accomplished only with some wash-out of deuterium, and decomposition of the nitroalkane by processes discussed later. For **4**, chiral HPLC of the ester, either before or after deuteration, yielded optically active material, which was then hydrolysed with no detectable loss of either label or of optical activity (Scheme 1).

Hydrolyses of nitronic acid tautomers of nitroalkanes to ketones or aldehydes (the Nef reaction^[52]) usually occurs at relatively high acidities^[53] (*ca* 3 M HCl) and is not normally

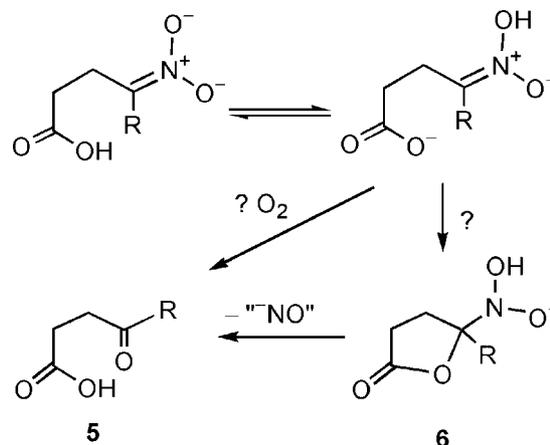


Scheme 1. Preparation and labelling of 4-nitropentanoic acid, **3**, and 4-nitro-4-phenylbutanoic acid, **4**

regarded as a complication in studies of deprotonations of simple nitroalkanes or in reversions of nitronic acid tautomers to nitroalkanes in near neutral aqueous medium. Lewis, however, noted that the anion of **3** was relatively unstable, decomposing to 4-oxopentanoic acid **5** ($R = \text{Me}$) with evolution of a gas presumed to be nitrous oxide.^[21,22] To account for the apparent occurrence of this Nef chemistry under the mildly basic conditions of his observations, he suggested that the carboxylate group might participate in forming the putative tetrahedral intermediate of the hydrolytic Nef reaction by nucleophilic attack on the carbon of the nitronate or nitronic acid^[54] to give a γ -lactone **6** as shown in Scheme 2.

Our first observations of the behaviour of aqueous solutions of 4-nitropentanoate confirmed the ready formation of **5**. Since the chemistry leading to **5** was not well understood, and might have complicated measurements of deprotonation rates, this aspect of the reactivity was briefly investigated further using **4** (rather than **3**) because of its more convenient UV-Vis properties. 1-Phenylnitropropane then serves as a reference compound lacking the catalysing intramolecular carboxylate group.

Solutions of phenylnitropropane (*ca* 10^{-5} M) in distilled but undegassed water exhibited the expected shoulder at 240 nm



Scheme 2. Nef chemistry on nitronates or nitronic acids carrying carboxylic acid residues

characteristic of the nitro group.^[47] Addition of aqueous sodium hydroxide then yielded a new peak, $\lambda_{\text{max}} = 275 \text{ nm}$ ($\epsilon_{\text{max}} = 6300 \text{ M}^{-1} \text{ cm}^{-1}$), with a shoulder to 225 nm, consistent with formation of the nitronate anion, and this basic solution was stable. Acidification to pH 1 immediately shifted the peak to 262 nm with an increased extinction, changes associated with formation of the nitronic acid tautomer, expected to have $\text{pK}_{\text{a}} \approx 4$.^[54] This spectrum, however, did not, as expected, revert cleanly to that of the original phenylnitropropane, instead slowly developing a new peak at 244 nm ($\epsilon_{\text{max}} 10300 \text{ M}^{-1} \text{ cm}^{-1}$). When changes were complete, GLC analysis of the solution and comparison with authentic samples confirmed recovery of phenylnitropropane and of propiophenone, the Nef product. In successive experiments, the ratio of propiophenone to phenylnitropropane was enormously variable, but it was clear that the production of the ketone was reduced by use of degassed materials and solvents, and enhanced by use of oxygenated water.

A solution of **4** (ca 10^{-4} M) in undegassed water similarly exhibited only a shoulder at 240 nm, and addition of dilute sodium hydroxide produced a new peak at 275 nm, associated formation of the nitronate, again stable for prolonged periods. On acidification, however, the solution did not yield any indication of a peak corresponding to the nitronic acid, but immediately showed a new absorption at 244 nm ($\epsilon_{\text{max}} 11000 \text{ M}^{-1} \text{ cm}^{-1}$) corresponding closely to that of 4-oxo-4-phenylbutanoic acid, **5** (R = Ph), the Nef product, whose presence was confirmed by subsequent isolation and comparison with authentic material. When solvents and reagents were degassed, acidification of the nitronate produced a peak at 266 nm consistent with formation of the nitronic acid. Ketone formation was suppressed, and **4** as well as **5** could then be recovered after the cycle of basification and re-acidification.

These observations are consistent with formation of ketonic products, not from a hydrolytic Nef reaction, but from a process involving reaction between dissolved oxygen and the nitronic acids, with the carboxylic acid in **4** facilitating the reaction. Observations on more concentrated solutions by $^1\text{H-NMR}$ spectroscopy support this conclusion. Solutions of the nitronate anion of **4** (10^{-2} M) were prepared by addition to an excess of sodium deuteroxide in undegassed 30:70 vol:vol: $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ mixture. Besides the phenyl signals, the spectrum showed 2H-triplets^[55] at δ 2.99 and at δ 2.04 and in this basic solution there was no indication of decay either to nitroalkane or to Nef product. Addition of 2 molar equivalents of CD_3COOD (relative to the sodium deuteroxide) induced an immediate small change in chemical shifts with distinct broadening of the signal at δ 2.99, and then evolution of the spectrum to that of a mixture of **4** and **5** (R = Ph) over a period of less than 5 min at 20°C . The amount **5** formed was compatible with consumption of the available oxygen in the NMR tube^[56] and, if air was bubbled through the tube immediately after acidification, **5** was the exclusive product. Repetition of the experiment with thoroughly degassed solutions, with addition of acetic acid, under argon, completely suppressed formation of **5** (R = Ph). When phenylnitropropane, was used similar results were obtained but the conversions were much slower, taking place over 45 min.

Oxidative cleavage of nitronates is a synthetically preferred method of converting nitroalkanes to carbonyl compounds,^[57] but with one exception,^[58] molecular oxygen has not been used as the oxidant, and the only *in vitro* reactions of dioxygen with nitronates reported are those of a nitronate-copper com-

plex.^[59,60] In contrast, oxidative denitrifications of nitroalkanes, catalysed by flavin-dependent enzymes of bacterial, fungal and plant origin^[60-63] consume molecular oxygen, and as we have noted earlier,^[44] at least one enzyme-catalysed denitrification involves a deprotonation of the nitroalkane by the carboxylate of an active site aspartate residue.^[44] There are interesting parallels here, and perhaps also some warnings for the interpretation of data on deprotonations of nitroalkanes when these have been monitored by UV-Vis spectroscopy. These observations will be pursued elsewhere; for the present, the important conclusion is that this oxidative Nef chemistry occurs after deprotonation of the nitroalkane and will not complicate measurements of deprotonation rates by racemization of optically active nitroalkanes.

Reaction kinetics

For direct comparability with the results of Lewis, the solvent chosen for the study of **3** was that used by Lewis, 54:46 (wt:wt) t-BuOH:water. To confirm conditions under which intramolecular deprotonations might be observed we initially examined racemizations in the presence of varying amounts of tetramethylguanidine (TMG), a strong base (for TMGH^+ , $\text{pK}_{\text{a}} = 13.6$)^[64] capable of deprotonating quantitatively both the carboxylic acid and the nitroalkane. Under these conditions, with undegassed solvents, material recovered from solutions at completion of the racemizations contained up to 20% of 4-oxopentanoic acid, **5** (R = Me).

With less than stoichiometric amounts of TMG, loss of optical activity was first order. With excess TMG, behaviour was complex, with a fast initial phase followed by a prolonged slow phase. In view of the complexity across the range, initial rates, ($d\phi/dt_{\text{t}} = 0$), were determined for all reactions and divided by initial rotations (ϕ_0) to yield comparable first order rate constants which are presented in Table 1 and graphically in Fig. 3.

The plot shows a break at base equivalence point linking two linear sections, and is consistent with the reaction sequence shown in Scheme 3, in which the chiral compound which we depict as a dibasic acid, $\text{HO} \sim \text{*CH}$, is first deprotonated stoichiometrically to yield the carboxylate, $\text{O}^- \sim \text{*CH}$. The kinetically significant processes are then deprotonations at the carbon of the nitroalkane. The intramolecular process (governed by k_{intra}) results in racemization, but, because the nitroalkane is at least 10^3 times less basic than the carboxylic acid, does not consume additional base. With excess base, deprotonations

Table 1. Initial rates for racemizations of 0.018 M 4-nitropentanoic acid, **3**, in 54:46 (wt:wt) t-BuOH:water at 30.9°C in the presence of tetramethylguanidine

[TMG]/[3]	$10^5 k/s^{-1}$
0.251	3.85
0.522	7.22
0.996	16.9
1.136	88.2
1.445	215
1.512	277
1.990	530
2.590	827

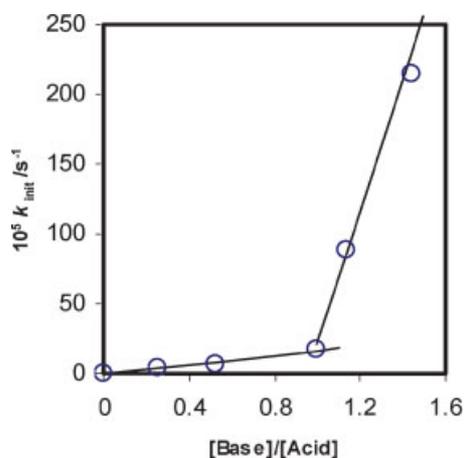
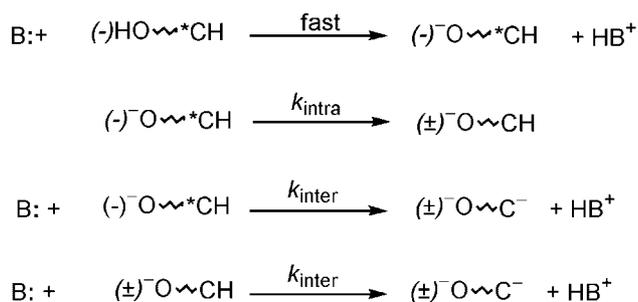


Figure 3. Effect of added tetramethylguanidine on rates of racemization of optically active 4-nitropentanoic acid **3** at 30.9°C in 54:46 (wt:wt) t-BuOH:water



Scheme 3. Reaction scheme for loss of optical activity in reaction of strong bases with **3** or **4**

governed by k_{inter} , yield a carboxylate-nitronate dianion, $\text{O}^-\sim\text{C}^-$, with consumption of an additional equivalent of added base.

The initial linear portion of the plot in Fig. 4 has the equation

$$10^5 \times k_{\text{init}} = 16.9(\pm 1.3) \times [\text{base ratio}] - 0.48(\pm 0.75) \quad (1)$$

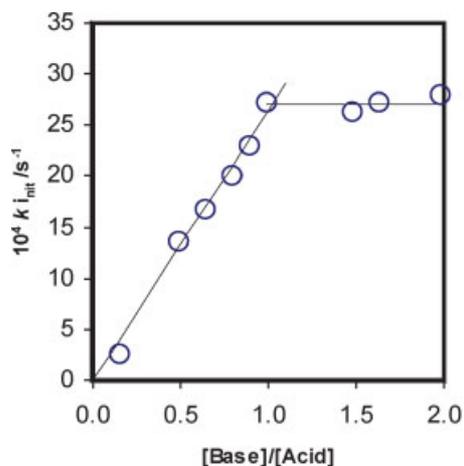


Figure 4. Effect of added triethylamine on rates of racemization of optically active 4-nitro-4-phenylbutanoic acid **4** at 30.04°C in 2:1 (wt:wt) DME:water

which describes the increase in rate proportional to the fraction of the **3** in its carboxylate form, and confirms the absence of significant competing water-catalysed reaction. The slope of the line, $16.9 \times 10^{-5} \text{ s}^{-1}$, is then the rate constant for the intramolecular reaction and in reasonable agreement with the value ($18.1 \times 10^{-5} \text{ s}^{-1}$) obtained by Lewis for the same reaction using pyridine as base.^[21,22] Lewis also obtained a value for the second order rate constant for acetate catalysed iodination of the methyl ester of 4-nitropentanoic acid^[65] in the same medium ($4.0 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$), and the ratio of first and second order rate constants gives the effective molarity, $\text{EM} \approx 4.3$, of the catalysing carboxylate in 4-nitropentanoic acid, a low value but comparable with those tabulated for other such intramolecular carbon acid deprotonations.^[66–68]

The much steeper line after equivalence point has the form:

$$10^5 \times k_{\text{init}} = 517(\pm 14) \times [\text{base ratio}] - 511(\pm 25) \quad (2)$$

and represents the contribution from proton abstractions by the excess strong base from the nitroalkane moiety of the carboxylate. Correction for initial NPA concentration gives a second order rate constant, $k_{\text{inter}} = 2.9 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$. Since TMG is sufficiently basic to generate some hydroxide in this medium, this rate constant will itself be a complex quantity reflecting contributions from both active bases.

For the reactions of **4** and its ester, the solvent mixture was changed to 2:1 wt:wt dimethoxyethane:water (28.6:71.4 mole ratio), to permit later use of D_2O in the experiments. Racemizations of **4** in this mixture were similarly examined, but with the TMG replaced by the less basic triethylamine ($\text{p}K_{\text{a}} = 11.01$ for Et_3NH^+), which, when in excess, was shown by separate UV–Vis observations to be capable of fully deprotonating both carboxyl group and the CH of the nitroalkane in this medium. For these reactions, losses of optical activity were first order for base ratios < 1 , and showed little deviation from first order behaviour with $1 < \text{base ratio} < 2$. For full comparability with the behaviour of **3** and TMG however, initial rates were determined as before and are presented in Table 2 and graphically in Fig. 4. Recovery of material from solutions at completion of the racemizations now yielded 4-nitro-4-phenylbutanoic acid **4**, with less than 2% of 4-oxo-4-phenylbutanoic acid **5** ($\text{R} = \text{Ph}$).

The plot of rate constant against base ratio shows that, with less than the full equivalent of base, rates are again proportional to the fraction of acid in its salt form with no indication of a

Table 2. Initial rates for racemizations of 0.023 M 4-nitro-4-phenylbutanoic acid **4**, in 2:1 (wt:wt) DME:water at 30.04°C in presence of triethylamine

$[\text{Et}_3\text{N}]/[\mathbf{4}]$	$10^4 k/\text{s}^{-1}$
0.148	2.50
0.493	13.6
0.642	16.8
0.790	20.2
0.888	23.0
0.987	27.2
1.480	26.3
1.628	27.2
1.974	27.9

significant water-catalysed process

$$10^4 \times k_{\text{init}} = 26.6(\pm 0.9) \times [\text{base ratio}] - 0.42(\pm 0.58) \quad (3)$$

The slope of the line gives the rate constant for the spontaneous reaction of the salt of **4**, $k = 26.6(\pm 0.9) \times 10^{-4} \text{ s}^{-1}$, some 15-fold faster than the reaction of **3**, reflecting both the change in medium, and the slightly enhanced acidity of the carbon acid associated with replacement of methyl by phenyl in the nitroalkane. In contrast to the behaviour of **3** with TMG, rates are little affected by excess of the weaker triethylamine showing, in terms of the reaction scheme (Scheme 3), that this base neither competes efficiently with the intramolecular carboxylate, nor generates sufficient hydroxide in this medium at the concentrations used for its effects to be obvious at the base concentrations used.

To estimate the effective molarity of the intramolecular carboxylate, racemizations of optically active methyl ester of **4** were measured also using buffered acetic acid ([HOAc]:[NaOAc] = 1.0). These were cleanly first order and the dependence of rates on concentration of sodium acetate (Table 3) is given by the relationship:

$$10^5 \times k_{\text{obs}} = 19.3(\pm 3.0) \times [\text{NaOAc}] + 1.5(\pm 0.6) \quad (4)$$

Comparing the second order rate constant for catalysis by acetate with that for spontaneous reaction of the carboxylate of **4** yields EM = 13.7, marginally larger only than that found for **3**.

Generally, the behaviours of **3** and **4** are closely comparable, but before moving to the primary kinetic isotope effects in **4**, we consider two possibly related complications to both measurement and modelling.

The first is that water might be involved as a proton shuttle in the intramolecular deprotonation, and this has been probed by measurement of the solvent isotope effect. Rates for racemizations of optically active **4** were measured in at 30.04 °C in a D₂O–DME mixtures, using triethylamine with [Et₃N]/[**4**] = 0.800. Loss of optical activity was first order, and duplicate runs gave $k = 28.6 (\pm 0.5) \times 10^{-4} \text{ s}^{-1}$. From Equation (3), the rate constant for reaction in an H₂O/DME mixture at the same base ratio is $20.8 \times 10^{-4} \text{ s}^{-1}$, giving $k^{\text{H}_2\text{O}}/k^{\text{D}_2\text{O}} = 0.73(\pm 0.04)$, for the intramolecularly catalysed process. Interpretation here is complicated a little by the use of mixed solvent, but this ratio is close to that reported for the deprotonations of 2-nitropropane by hydroxide in water^[28] ($k^{\text{H}_2\text{O}}/k^{\text{D}_2\text{O}} = 0.68$), and to the fractionation factor for isotopic distribution between bulk water and water hydrogen-bonded to oxy-anionic base ($\phi = 0.74$).^[69] The inverse effect is interpreted in terms the stronger DOD...OR bonds compared to HOH...OR, and a requirement for solvation of the oxy-anionic base to be reduced prior to its

involvement in deprotonation of the nitroalkane. The effect is not compatible with involvement of solvent derived hydron in covalent bonding changes in the rate-limiting step.

The second was that some exchange of nitroalkane proton with solvent might occur independently of the racemization (i.e. $k_{\text{exchange}} > k_{\text{racemisation}}$),^[70] possibly by a discrete transient pyramidal hydrogen-bonded carbanion of the type suggested by Bordwell *et al.*^[71] Such exchange has been shown to lead to erroneously low values for k^{D} in reactions of deuteriated isotopomers.^[72] The excellent first order behaviour observed in racemizations in the D₂O/DME mixture in the determinations solvent isotope effect militates against this, but exchange reaction of **4** in D₂O/d₁₀-DME was also monitored directly by ¹H-NMR spectroscopy to follow loss of the signal associated the nitroalkane hydrogen (δ 5.6). With one equivalent of triethylamine, the first order rate constant at 30.1 °C was $33.2 (\pm 2.8) \times 10^{-4} \text{ s}^{-1}$, giving an apparent $k_{\text{exchange}}/k_{\text{racemisation}} = 1.25$. However, allowance for the solvent isotope effect associated with replacement of H₂O in the racemization experiments by D₂O in the NMR experiment ($k^{\text{H}_2\text{O}}/k^{\text{D}_2\text{O}} = 0.73$) reduces the ratio to $0.91(\pm 0.14)$, and we conclude that, within the combined experimental uncertainties, $k_{\text{racemisation}} = k_{\text{exchange}}$ in these reactions.

Primary kinetic isotope effects

The practical difficulties in measuring kinetic isotope effects in these racemizations are associated with ensuring that the isotopomeric reactants experience identical conditions of solvent, temperature and degree of neutralisation. To achieve this, we have resolved partially deuteriated mixtures of isotopomers and then monitored the course of racemization of these isotopically mixed samples, catalysed by less than a full equivalent of TMG, in the case of **3**, and Et₃N in the case of **4**. This procedure assumes firstly that γ -isotopic substitution has no significant effect on the acidity of the carboxylic acid, and secondly, that the substitution does not significantly affect the optical activity. For the first, we note that the effects at the α -deuteriation on acidity of carboxylic acids are small ($K_{\text{a}}^{\text{CH}_3}/K_{\text{a}}^{\text{CD}_3} = 0.03$ in acetic acid)^[73–75] and suggest that the effects of isotopic substitution even more remote from the carboxylate can safely be ignored. For the second, the resolution and measurement of optical activity in compounds such PhCHDCH₃^[76] shows there must indeed be an effect from isotopic substitution, but the associated changes in specific rotations are very small compared to those for the compounds used, and we believe that this effect can also be safely ignored.

With these simplifying assumptions, the optical rotation, ϕ_t , of the partially neutralised isotopic mixture is expected to decay following relationship (5), where ϕ_{inf} is the optical rotation at infinite time:

$$\phi_t = \phi_{\text{(inf)}} - \text{Fr}_{(\text{H})} \Delta\phi e^{-k_{\text{H}}t} - (1 - \text{Fr}_{(\text{H})}) \Delta\phi e^{-k_{\text{D}}t} \quad (5)$$

The fraction here ($\text{Fr}_{(\text{H})}$) is the fraction undeuterated within the optically active component, and not in the total mixture which may contain racemic material, both labelled and unlabelled, but which is 'transparent' to polarimetry. The relationships then sums the contributions from each optically active isotopomer to the total optical rotation of the mixture, and rates, extracted from time dependences of the rotations by non-linear regression,^[77,78] are presented in Table 4, along with the associated activation parameters.

Table 3. Rate constants for racemizations of methyl 4-nitro-4-phenylbutanoate in 1:1 NaOAc:HOAc buffered 2:1 DME: water at 30.04 °C

[NaOAc]	$10^5 k/\text{s}^{-1}$
0.140	4.32
0.210	5.30
0.282	7.05

Table 4. Primary kinetic isotope effects for base-catalysed racemizations of carboxylic acids of **3** and **4**, and of the methyl ester of **4**

T (°C)	$10^4 k^H/s^{-1}$	k^H/k^D	Activation parameters
Acid 3 in t-BuOH: H ₂ O. Salt formation with TMG.			
30.9	1.69 (±1.3)	5.68 (±0.17)	$\Delta G_{303}^{\ddagger H} = 88.6(\pm 3.8) \text{ kJ mol}^{-1}$
Acid 4 in DME:H ₂ O. Salt formation with Et ₃ N.			
20.16	11.6 (±0.3)	5.99 (±0.28)^a	$E_a^D - E_a^H = 5.5(\pm 0.1) \text{ kJ mol}^{-1}$, $A^H/A^D = 0.63(\pm 1.03)$
32.04	35.5 (±0.8)	5.51 (±0.06)^a	$E_a^H = 67.6(\pm 1.2) \text{ kJ mol}^{-1}$, $A^H = 1.68(\pm 1.2) \times 10^9$
50.02	151 (±2.5)	4.87 (±0.12)^a	$\Delta H^{\ddagger H} = 65.1(\pm 1.2) \text{ kJ mol}^{-1}$, $\Delta S^{\ddagger H} = -78.8(\pm 3.8) \text{ J K}^{-1} \text{ mol}^{-1}$
25.0	18.5*	5.78*	$\Delta G_{298}^{\ddagger H} = 88.6(\pm 3.8) \text{ kJ mol}^{-1}$
Methyl ester of 4 in DME:H ₂ O in presence of 0.25 M NaOAc			
30.40	4.18 (±0.01)	7.18 (±0.09)	
50.00	21.1 (±0.02)	6.33 (±0.17)	$E_a^D - E_a^H = 5.2 \text{ kJ mol}^{-1}$, $A^H/A^D = 1.08$
25.0	2.58*	7.43*	

^a Mean of two experiments.
* Extrapolated values.

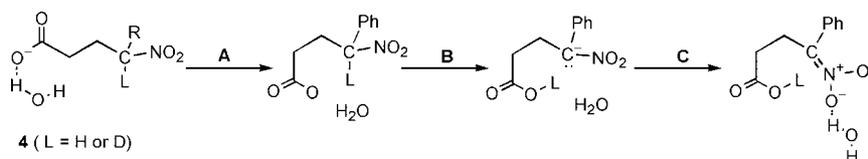
Conclusions

Taken together, the combined results of the studies described above are consistent with the racemizations of salts **3** and **4** involving a direct transfer of proton from the nitroalkane carbon to an anionic oxygen of the intramolecular carboxylate via a six-centre cyclic array, and that rates of racemization are a reliable measure of deprotonation rates. The kie found in this work for the racemization 4-nitropentanoic acid, **3** ($k^H/k^D = 5.68 \pm 0.17$ at 31°C) is satisfyingly close to that reported by Lewis for deprotonations of 4-nitropentanoic acid, using iodine-trapping methods ($k^H/k^D = 5.5 \pm 0.5$). Replacement of methyl by phenyl, and change of solvent from aqueous t-BuOH to aqueous dimethoxyethane makes little difference with $k^H/k^D = 5.51 \pm 0.06$ at 32°C for racemizations of **4** in aqueous dimethoxyethane.

In considering the detailed course of the reaction (Scheme 4), we assume that ground state conformation of the reactant in the medium is the extended one shown, minimizing torsional interactions along the chain, and expect that the anionic oxygens of the carboxylate are solvated, with hydrogen bonding with a number of water molecules,^[79] although we show only one here. Before the intramolecular proton transfer can occur, there must be conformational adjustment to bring the basic carboxylate group within reacting distance of the nitroalkane hydrogen, and desolvation of the oxyanion (Step A). Since the transfer occurs through a six-centre cyclic array, the energy increase associated with the conformational change is likely to be only a little greater

than that in the a staggered-to-gauche change in butane (3.8 kJ mol⁻¹). If the effects on the pK_a of carboxylic acids of moving from water to DMSO (4.8 in water to 12.3 in DMSO^[80]) are taken to reflect the effects of desolvation on the stability of the anionic base in a polar non-aqueous environment, then the energetic cost of the desolvation may be as much as 42.8 kJ mol⁻¹, so that half of the measured free energy barrier might be accounted for by processes not linked directly to the proton transfer which occurs in step B, with no water molecule having more than 'interested-spectator' status. This may lead to a carbanion in which charge remains largely localised on carbon,^[71] and the process is then completed by charge and solvent re-organisation to yield the planar, optically inactive nitronate in step C. The extent of linkage between the proton motion and the secondary re-organisation remains to be established, but the fact of a substantial kinetic isotope effect and the equality of exchange and racemization rates demands that step C is substantially faster than the reverse of step B.

Table 4 includes Arrhenius and Eyring parameters for the H-isotopomer of **4**, and we note that the reaction exhibits a large negative entropy of activation. The kie for the intramolecular reaction in **4** does not differ significantly for that found for **3**, and the temperature dependence yields Arrhenius parameters indicating that the rate ratios are associated with differences in activation energies ($E_a^D - E_a^H$) rather than in pre-exponential factors (A^H/A^D), and thus not consistent with major tunnelling contributions.

**Scheme 4.** Separation of steps in the racemizations of the salts of **4** (L = H and D)

Notably, the *k*_{ie} for the intramolecular reactions is significantly smaller than that catalysed intermolecularly by acetate (this work, 7.2 at 25°C), but again, the intermolecular kinetic isotope effect is associated with differences in activation energy ($E_a^D - E_a^H$) rather than in pre-exponential factors. The smaller intramolecular effect may be associated with some stiffening of isotopically sensitive modes in the cyclic transition state, and indeed, may be signalling that the six-membered ring is not an ideal size for accommodating a proton transfer. Studies on intramolecular hydrogen bonding using diamides^[81] have suggest that rings as large as nine may be necessary to accommodate fully relaxed linear hydrogen bonds.

These measurements on a well-defined intramolecular process will be used to test and parametrise computational methods which can then be applied to much more complex reacting systems, and the results of a parallel computational study will be reported separately.

EXPERIMENTAL SECTION

General methods

NMR spectra were recorded on Varian Unity INOVA-300 or Bruker 500 AMX spectrometers. Chemical shifts are parts per million (ppm) downfield from tetramethylsilane. Signal splittings are quoted as: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Infrared spectra were recorded on an ATI Mattson Genesis Series FTIR and were run as liquid films or as films evaporated from deuterated chloroform. UV-Vis spectra were recorded on a Cary 50 UV-Visible spectrophotometer. Mass spectra were recorded on a Kratos MS25, Fisons VG Trio 2000 or on a Micromass platform. Modes of ionisation are electron impact (EI), positive chemical ionisation (+CI) using ammonia and fast atom bombardment (FAB), positive electrospray (+ES) and negative electrospray (-ES). Melting points were recorded on a Kofler heated stage microscope and are uncorrected. Thin layer chromatography (TLC) was carried out on Polygram Sil G/UV₂₅₄ 0.25 mm silica gel plates with solvent systems as indicated. Analytical gas chromatography was carried out using: Perkin-Elmer capillary gas chromatography model 8310 with flame ionisation detection (FID). Analytical HPLC was carried out using: Waters 510 HPLC pump, Bondclone C18 100 × 8 mm column, Perkin-Elmer LC480 Diode Array System with detection at 255 and 285 nm pre-mixed degassed solvent systems 75:25 MeOH:H₂O or as stated in the text, flow rate 2 ml min⁻¹.

The compounds

Phenylnitropropane

Propiophenone oxime was oxidised with sodium perborate in acetic acid according to the method of Olah *et al.*^[82], to give phenylnitropropane in 45% yield after distillation; b.p. 72°C at 2 mm Hg (lit 75°C at 1 mm Hg).

Methyl 4-nitropentanoate

AmberlystTM A-21 (5.00 g), nitroethane (6.0 g) and methyl acrylate (5.81 g) were mixed and the solution left stirring overnight at 40°C. The reaction was monitored by NMR and on completion the mixture was cooled and filtered. The resin was washed with a little dichloromethane, and the combined liquids dried over sodium sulphate before removal of solvent under reduced

pressure to yield 8.9 g of yellow oil which was purified by bulb-to-bulb distillation (100°C at 1 mm Hg). δ H (300 MHz, CDCl₃) 4.68 (1H, m, 14 lines, W = 35 Hz), 3.72 (3H, s), 2.43 (2H, m, 13 lines, width = 57 Hz), 2.29 (1H, m, 14 lines, width = 37 Hz), 2.12, (1H, m, 12 lines, W = 34 Hz), 1.61 (3H, d, J = 8 Hz); δ C (75 MHz, CDCl₃) 172.5, 89.9, 82.5, 51.9, 34.0, 29.9, 28.728, 21.6, 19.16; $\nu_{\max}/\text{cm}^{-1}$, 2995, 2955, 1743, 1543, 1554, 1438.

4-Nitropentanoic acid, **3**

Methyl 4-nitropentanoate (4.1 g) and 2:1 vol:vol TFA:water (100 ml) were heated at 80°C overnight in an RB flask before removal of solvents under reduced pressure by rotary evaporation. Toluene (30 ml) was added to the residue in the flask, and then also evaporated under reduced pressure to leave a white crystalline solid, recrystallised for ether:petroleum (3.54 g). mp 33°C (lit., 34.532^[51]). δ H (300 MHz, CDCl₃) 11.5 (1H, s), 4.85 (1H, m, 14 lines, W = 35 Hz), 2.44 (2H, m, 13 lines, width = 57 Hz), 2.24 (1H, m, 14 lines, width = 37 Hz), 2.07, (1H, m, 12 lines, W = 34 Hz), 1.77 (3H, d, J = 8 Hz); δ C (75 MHz, CDCl₃) 177.9, 89.9, 82.6, 30.3, 29.0, 19.05; $\nu_{\max}/\text{cm}^{-1}$, 2583 (b), 2994, 1712, 1548, 1450, 1416. *m/z* (Cl⁺) 179 (51%, M + NH₄⁺) 162 (100%, M + H⁺), 146,(64%).

Methyl 4-deuterio- 4-nitropentanoate, **3(L = D)**

Methyl 4-nitropentanoate (2.0 g) was dissolved in methanol-OD (10 ml) and pyridine (1 ml) added. The reaction mixture was heated to 60°C and the exchange was followed by ¹H-NMR spectroscopy until appropriate deuterium incorporation, by integration of the signal at δ 4.68. The solvent was then removed under reduced pressure, the residue taken up in ether, and the pyridine removed with successive washes with dil. hydrochloric acid. The ethereal solution was then dried over sodium sulphate, before evaporation and distillation as before.

4-Deutero-4-nitropentanoic acid, **3(L = D)**

Methyl-4-deutero-4-nitropentanoate (2.0 g) was hydrolysed by overnight treatment with 2:1 water:TFA mixture at 80°C and isolation as described above to give crystals, (1.75 g). δ H (300 MHz, CDCl₃) 11.5 (1H, s), 2.44 (2H, m, width = 50 Hz), 2.24 (1H, m, 6 lines, width = 31 Hz), 2.07, (1H, m, 6 lines, W = 30 Hz), 1.70 (3H, s); δ C (75 MHz, CDCl₃) 177.9, 89.9, 82.6, 30.3, 29.0, 19.05; $\nu_{\max}/\text{cm}^{-1}$, 2583 (b), 2994, 1712, 1548, 1450, 1416.

Resolution of 4-nitropentanoic acid and 4-deutero-4-nitropentanoic acid

4-Nitropentanoic acid was resolved by crystallisation of its quinine salt as described by Theilacker and Wendtland.^[51] The acid (1.0 g) was dissolved in acetone (10 cm³) with gentle warming. Quinine (2.1 g) was also dissolved in acetone (10 cm³), and then the two warm solutions mixed and allowed to stand until no more crystals formed. The mother liquor was then pipetted away and the crystals washed with a little cold acetone. The mother liquor was retained for subsequent crops. The solid was then dissolved in the minimum amount of methanol (5 cm³) and sulphuric acid solution (20 cm³ of 20%) added. The resolved compound was extracted into ether (30 cm³) which was dried over sodium sulphate before evaporation of solvent to leave a clear oil which slowly crystallised, (0.42 g, $[\alpha]_D = 13^\circ$ in acetonitrile). Similar treatment of 4-deutero-4-nitropentanoic acid yielded crystals (0.42 g, $[\alpha]_D = 9.6^\circ$ in acetonitrile). Integ-

ration of the signals at δ 4.85 and at δ 1.70 yielded estimate of the deuterium incorporation in samples used in isotope effect measurements.

Methyl 4-nitro-4-phenylbutanoate

This ester was prepared by the method of Ballini and Bosica.^[48] Amberlyst-A21TM resin (1.2 g) was added to a stirred mixture of methyl acrylate (1.1 g, 12.8 mmol) and nitrophenylmethane^[83] (1.42 g, 10.2 mmol), under nitrogen. After stirring overnight at 25°C, the resin was separated from the liquid and extracted 3 times with 20 ml portions of ether. The liquid and combined ether extracts were then dried over anhydrous sodium sulphate, and the ether was removed under vacuum yielding methyl 4-nitro-4-phenylbutanoate as a clear oil (1.8 g, 78.3%), of sufficient purity for use in subsequent hydrolysis. Distillation of a portion yielded material, b.p. 134°C at 1.4 mm of Hg (lit., 205°C at 0.4 mm); δ H (300 MHz, CDCl₃) 7.44 (5H, m), 5.65 (1H, dd, $J = 5.8$, $J = 8.5$), 3.73(3H, s), 2.82(1H, m, 14 lines, width 34 Hz), 2.55–2.37 (3H, m); δ C (75 MHz, CDCl₃) 172.1, 133.9, 129.9, 127.9, 127.6, 89.9, 51.8, 30.0, 28.8; ν_{\max} (film)/cm⁻¹, 1737(s), 1552; m/z (CI) 241 (100%), 177; (EI) 177, 117; (ES-) 222(M-1, 20%), 208(100%).

Deuteration of methyl 4-nitro-4-phenylbutanoate

Methyl 4-phenyl-4-nitrobutanoate, (0.5 g, 2.25 mmol) was added to a 25 ml round bottom flask containing of methanol-D (4.0 cm³) and of pyridine (1.0 cm³). This was left for 24 h at 50°C, then the methanol and pyridine were removed under vacuum. The process was then repeated with fresh reagents. This yielded an oil, which was purified by bulb-to-bulb distillation yielding a clear oil, methyl 4-deuterio-4-nitro-4-phenylbutanoate (0.454 g, 2.03 mmol, 90.4%). Its proton NMR spectrum was closely similar to the all-H material, with diminution of the signal at δ 5.65, consistent with 77% deuteration; ν_{\max} (film)/cm⁻¹, 1738(s), 1552; m/z (CI) 242(100%), 178(65%); (EI) 178(15%), 118(100%), 105(80%), (ES-) 236(100%), 222(M-1, 90%).

Resolution of Methyl 4-nitro-4-phenylbutanoate and Methyl 4-deuterio-4-nitro-4-phenylbutanoate

Optically active methyl 4-nitro-4-phenylbutanoate was obtained by chromatography on a 25 × 1 cm ChiracelTM OD-H column eluting with 1% 2-propanol in hexane at 4 ml min⁻¹. A full separation of enantiomers was not achieved but judicious cutting of the peaks yielded samples with $[\alpha]_{365}$ up to 75° for dichloromethane solutions.

4-Nitro-4-phenylbutanoic acid and 4-deuterio-4-nitro-4-phenylbutanoic acid, **4** ($L = H$ and D)

Methyl 4-nitro-4-phenylbutanoate (70 mg, 0.315 mmol) and 2:1 v:v TFA:D₂O solution (2 cm⁻³) was placed in a screw-top test tube which was heated at 55°C for 12 h, with reaction monitoring by periodic ¹H-NMR spectroscopy. Removal of volatiles under vacuum yielded 4-nitro-4-phenylbutanoic acid as a waxy solid (48 mg), mp 78–83°C and material used in measurements was purified by crystallisation from petrol giving white needles, mp 94–98°C.

For all-H material, δ H (300 MHz, CDCl₃) 7.44 (5H, m), 5.65 (1H, dd, $J = 5.8$, $J = 8.5$), 2.82(1H, m, 14 lines, width 36 Hz), 2.55–2.37 (3H, m); δ C (75 MHz, CDCl₃) 177.1, 133.7, 130.0, 129.1, 127.6, 89.7,

29.8, 28.5; ν_{\max} (film)/cm⁻¹, 1703(s), 1552; m/z (CI) 227, 180(100%); (EI) 163, 117(100%); (ES-) 208(M-1, 100%), 113(40%).

For the deuterated material the ¹H-NMR spectrum was closely similar to the all-H material, with diminution of the signal at δ 5.65, consistent with 77% deuteration; ν_{\max} (film)/cm⁻¹, 1715, 1552; m/z (CI) 228(75%), 181(100%); (EI) 164(65%), 118(100%).

Sensitivities to oxygen

Observations by UV-vis spectroscopy were on a Cary 50 Bio Spectrometer. NMR experiments used a 400 MHz INOVA instrument with probe held at 20°C.

To a NMR tube containing 1 μ l of phenylnitropropane and 0.5 μ l of pivalic acid as internal reference was added CD₃CN (230 μ l) and standardised NaOD in D₂O (460 μ l of 0.0906 M). For initial experiments no precautions for deoxygenation were taken. The NMR spectrum and then d₄-acetic acid (4.5 μ l) was injected into the tube to give a solution with pD of 5.6 at 25°C. Spectra were then collected. For reactions without oxygen, the tube was fitted with a subbaTM seal and nitrogen was bubbled slowly for 1 h through the solution via syringe needle before addition of the phenylnitropropane and pivalic acid and NaOD, and then again before acidification. Similar experiments were carried out with tubes containing a weighed amount (0.0011 g) of 4-nitro-4-phenylbutanoic acid in CD₃CN (230 μ l) and standardised NaOD in D₂O (460 μ l of 0.0891 M). Results are described in the text.

Reaction kinetics

Polarimetry

Optical rotations were monitored using a Perkin-Elmer PE 341 polarimeter. Reactions were run in a thermostatted cell whose temperature was monitored at the cell using a platinum resistance thermometer. Cell temperatures were constant over the course of a kinetic run to $\pm 0.02^\circ\text{C}$. Cell volume was 5 ml and the path length was 10 cm. The recorder voltage from the polarimeter and the output of the platinum resistance thermometer were taken via a Pt100 4-channel analogue-to-digital converter (from Pico Technology Limited) directly to a standard PC and logged continuously (1 reading per second) using the supplied data logging software (Picolog). Reactions were monitored for at least five half-lives (of the slower component when mixtures of isotopomers were used). Data was transferred to Microsoft Excel for treatment, usually non-linear least squares fitting to the appropriate function using the Solver add-in. Uncertainties and statistics associated with fitting were generated using the *SolvStat* macro of Billo.^[84] For fitting to Equation 5 in the text to extract kinetic isotope effects, there is a strong covariance between the k^H/k^D and the fraction of deuterated material, $F_{r(H)}$. Initial fitting was therefore carried out with the value of $F_{r(H)}$ fixed at that determined by NMR analysis of the sample of **3** or **4** used. Fitting was then repeated with $F_{r(H)}$ treated as a fully adjustable parameter. Data were only deemed acceptable if relaxing the restriction on $F_{r(H)}$ did not significantly change its value or any of the other derived parameters.

Racemizations of 4-nitropentanoic acid, **3**

For racemizations of 4-nitropentanoic acid, a stock solution of optically active 4-nitropentanoic acid (0.181 M) in 54:46 wt:wt t-BuOH: water was prepared, having an optical rotation

$\phi = -3.411^\circ$ (at 598 nm and 30.9°C). These solutions showed slow loss of optical activity on storage (presumably via the small equilibrium concentration of the carboxylate) and stored solutions were acidified ($[\text{HCl}] = 2.5 \times 10^{-3} \text{ M}$) to suppress ionisation of the carboxylic acid. For the rate measurements, weighed quantities of TMG (0.103 g, 0.208 g, 0.026 g, 0.206 g, 0.1501 g, 0.157 g, 0.054 g, 0.1176 g) were added with shaking to 5 ml of the stock solution in a volumetric flask, which was thermostatted to the same temperature as the polarimeter cell.

Primary kinetic isotope effects in racemizations of 4-nitropentanoic acid, **3**

Studies were conducted at 30.9, 41.1, and 49.3°C. Separate stock solutions of weighed amounts of optically active partially deuteriated 4-nitropentanoic acid and of TMG in 54/46 w/w tert-Butanol/water were prepared. Concentrations varied between 0.68 and 0.15 M for the acid and 0.6 and 0.08 M for the TMG, with concentrations chosen so that mixing would yield solution of partially neutralised acid with a known acid:base ratio, adjusted to give a conveniently observable rate. Before mixing, these were brought to the reaction temperature and then 2.5 cm³ of each solution transferred to a thermostatted flask for mixing before transferring by cannula to the cell as quickly as possible. Data logging zero time was taken from the time of mixing, and changes in optical activity at $\lambda = 598 \text{ nm}$ were monitored for at least $30 \times t_{1/2}$ anticipated for the H-isotopomer.

Material was recovered from these racemizations by acidification of the solutions with dil. hydrochloric acid, and extraction with dichloromethane. Examination by ¹H-NMR spectroscopy showed, in all cases, the presence of laevulinic acid (up to 20% of total) as well as 4-nitropentanoic acid.

Racemizations of 4-nitro-4-phenylbutanoic acid, **4**

The reaction medium was 2:1 wt:wt mixture of dimethoxyethane:water, made up using dimethoxyethane freshly distilled from calcium hydride and distilled deoxygenated water, and stored in the dark under nitrogen. Stock solutions of optically active 4-nitro-4-phenylbutanoic acid (0.24 g in 50.5 ml) and a more concentrated solution of triethylamine (0.86 g in 15.0 ml) were made up, under nitrogen, and sonicated briefly before mixing under nitrogen. Typically, an aliquot of the acid solution (5.0 ml) was placed in a stirred flask thermostatted to the reaction temperature (30.06°C) and a volume of the triethylamine solution added by micrometer syringe as rapidly as possible. The mixed solution was then transferred by cannula to the polarimeter cell, and changes in optical rotation monitored at $\lambda = 365 \text{ nm}$. Data logging was initiated at time of mixing of acid and basic solutions. For a series of measurements the acid:base ratio was varied and initial rotations were between 0.28 and 0.26°.

For the solvent isotope effects, stock solutions of optically active (0.276 g in 20.0 ml) and of triethylamine (0.105 g in 25 ml) were made up in a mixture of DME (102.6 g) and D₂O (56.4 g). For the reaction, these solutions were separately equilibrated to temperature and equal volumes (2.6 ml) mixed at the reaction temperature before transfer to the cell as before. Changes in optical rotation monitored at $\lambda = 365 \text{ nm}$, where initial rotations were ca 0.16°.

Material was recovered from these racemizations by acidification of the solutions with dil. hydrochloric acid, and extraction into dichloromethane. Examination by ¹H-NMR spectroscopy

showed 4-nitro-4-phenylbutanoic acid and no more than 2% of 4-oxo-4-phenylbutanoic acid.

Kinetics of deuteration of 4-nitro-4-phenylbutanoic acid, **4**, by NMR

The solvent was a mixture of d₁₀-DME (2.91 g) and D₂O (1.45 g). 4-Nitro-4-phenylbutanoic acid (0.032 g) was dissolved in the solvent mixture (0.75 ml), and transferred to an NMR tube. After equilibration to probe temperature, an aliquot of triethylamine was added by micrometer syringe, the tube shaken quickly and then returned to the probe. Spectra were then recorded at appropriate intervals, with at least 10 s between acquisitions. The probe temperature was measured before and after the experiment using signal separations in the spectrum of ethylene glycol. Decrease in ratio of integrals of signal at $\delta 5.45$ to the isolated signal of a non-exchanging hydrogen at $\delta 2.28$ with time was first order. Base ratios were measured by comparing integrals from the methyl signal of the triethylamine with that at $\delta 2.28$. In two separate experiments rate constants were $31.7(\pm 2.8) \times 10^{-5} \text{ s}^{-1}$ (at 28.4°C) and $34.7(\pm 2.8) \times 10^{-5} \text{ s}^{-1}$ (at 31.7°C) for fully neutralised materials. Uncertainties take into account the standard deviation of the fit, and in the base ratio.

Primary kinetic isotope effects in racemizations of 4-nitro-4-phenylbutanoic acid, **4**

Separate stock solutions of optically active partially deuteriated 4-nitro-4-phenylbutanoic acid (0.221 g in 45.1 ml) and a more concentrated solution of triethylamine (0.86 g in 15 ml) were made up in the 2:1 DME:H₂O solvent mixture, as before. The acid solution (5.0 ml) was placed in a stirred flask and equilibrated to the reaction temperature. Triethylamine solution (0.1 ml) was then added by micrometer syringe as rapidly as possible for mixing before transfer by cannula to the cell. Data logging zero time was taken from the time of mixing, and changes in optical activity at $\lambda = 365 \text{ nm}$ were monitored for at least $10 \times t_{1/2}$ anticipated for the H-isotopomer.

Racemizations of the methyl ester of **4**

Three acetate buffers (1:1 HOAc:NaOAc) in 2:1 DME:water were prepared with $[\text{NaOAc}] = 0.28, 0.42$ and 0.56 M . Equal volumes (2.5 ml) of each buffer and a stock solution of 4-nitro-4-phenylbutanoic acid (0.13 g in 12 g) were equilibrated to temperature and mixed before transfer by cannula to the cell, and changes in optical activity at $\lambda = 365 \text{ nm}$ were monitored for at least $5 \times t_{1/2}$.

For the kinetic isotope effects in the racemization, separate stock solutions in 2:1 DME:water of optically active partially deuteriated 4-nitro-4-phenylbutanoic acid (0.223 g in 50 ml) and an acetate buffer (AcOH 0.752 g, 0.0125 mol, and NaOAc, 1.025 g, 0.0125 mol, in 25 ml) were made up. The solutions were separately equilibrated to temperature and equal volumes (2.6 ml) mixed at reaction temperature (30°C or 50°C) before transfer by cannula to the cell. Data logging zero time was taken from the time of mixing.

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