Kinetics of the Detritiation of Carbon Acids Containing More than One Exchangeable Site

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A procedure has been established for measuring rate constant ratios for base-catalysed detritiations in compounds containing two exchangeable sites. A computer method for kinetic data treatment was calibrated by using mixtures of two acetyl-labelled acetophenones of known specific rates of detritiation. This was then applied to compounds containing two exchangeable sites as represented by an unsymmetrical ketone (**1**), a camphor (**2**), and a steroid (**3**). The method could be of value in providing direct information on transition state structure in proton transfer processes from sites differing in structural and stereoelectronic environments.

Many organic compounds contain more than one kind of labile carbon–hydrogen bond and such bonds are expected to undergo isotopic exchange at different rates.[1] The most common situation arises with unsymmetrical ketones, such as methyl ethyl ketone, where the two types of α hydrogen atoms have been found to exhibit different reactivities in base-catalysed H/D exchange.[2] Non-equivalence in carbonyl compounds can also arise from a differing stereochemistry imposed on two methylene protons which form part of a rigid ring system.[3,4] Molecules such as benzyl methyl sulphoxide contain non-equivalent methylene hydrogens by virtue of being diastereotopic and these hydrogens have also been found to undergo base-catalysed exchange at different rates.[5–8] Certain nitroaromatic compounds such as 1,3-dinitrobenzene and 1,3-dinitronaphthalene possessing aryl hydrogens of differing reactivities have similarly been examined.[9]

The H/D exchange processes in such systems have generally been followed by ^{1}H NMR spectroscopy. More recently, as an alternative to studying the rates of detritiation of specifically labelled compounds, a detritiation technique has been applied to various simple unsymmetrical ketones labelled at a number of exchangeable sites,[10] and at the *exo* and *endo* hydrogens in a number of camphors.[11] This method can also be used to estimate the pK_a values of alternative acidic sites in carbon acids.[12] The method used is similar to that presented here but suffers from the drawback that the position and extent of labelling is not known with any confidence. The importance of concurrent isotope exchange in biochemical systems possessing differing reactive sites has been demonstrated by Cooper and Abeles,[13,14] and by Johnston,[15] in their studies on the action of various enzymes or simple amino acids. Here we report on a new development in which ^{3}H NMR spectroscopy,[16] which allows one to measure the position and extent of labelling, is combined with the more traditional detritiation procedure. The validity of the method was established by using mixtures of two acetyl-labelled acetophenones whose rates of detritiation have been ascertained separately. In the latter case radio-gas chromatography[17] as well as ^{3}H NMR spectroscopy was used to determine the relative proportion of the two labelled ketones.

Results and Discussion

The kinetic data were analysed by means of a computer technique and occasionally supplemented by a more limited graphical procedure.[9] In the computer technique a program KIN 2 has been used to carry out a multivariate optimisation by the 'search' method of function evaluation.[18] The kinetic system is described in terms of a set number of parameters which are then adjusted such that a given criterion is obeyed. For systems in which the sampled data are not only a function of controlled parameters but also time, it is the 'least-squares error' criterion, S, that is most often used. This is defined in equation (1),

$$S = \sum_i^n (P^i_{cal} - P^i_{obs})^2 \quad (1)$$

where n is the number of data points and P_{obs}, the experimental extent of detritiation, given by equation (2), increases from zero

$$P_{obs} = \frac{N_0 - N_t}{N_0 - N_\infty} \quad (2)$$

to unity as detritiation proceeds. N_0, N_t, and N_∞ are the specific radioactivity values associated with the carbon acid at times $t = 0$, t, and at the end of the reaction, respectively. P_{cal}, the theoretical extent of detritiation, is given by equation (3),

$$P_{cal} = x_A[1 - \exp(-k_a t)] + x_B[1 - \exp(-k_b t)] \quad (3)$$

where x_A and x_B are the tritium atom fractions and k_a and k_b the unknown first-order rate constants for exchange from sites A and B. As x_A and x_B can be determined (from either ^{3}H NMR spectroscopy or radio-gas chromatography studies) the problem is therefore reduced to a two-parameter optimisation procedure.

Initially two acetyl-labelled acetophenones (the relative proportions could easily be varied) were chosen whose rates of detritiation were known[19] to be sufficiently different as to allow a graphical separation of the two detritiation rate constants. This was then followed by studies on mixtures where the rate ratios were much closer to unity and where the computer program was essential. Finally, the procedure was used for three types of compound where, for different reasons, two sites of exchange existed. These were represented by the unsymmetrical ketone, methyl neopentyl ketone (**1**), camphor (**2**), and the steroid dehydroepiandrosterone (**3**).

For the tritiated acetophenone mixtures the relative proportions were determined both by radio-gas chromatography and ^{3}H NMR spectroscopy, the agreement being well within 5%. At

Table 1. Rate constants ($k^T_{OH^-}/10^{-3}$ dm^3 mol^{-1} s^{-1}) for acetophenone mixtures at 25 °C as determined by graphical and computer procedures.

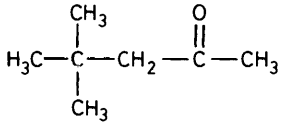
Ketone mixture	x_A [a]	$10^3 k^T_{OH^-}$ [b]	Rate ratio
(i) Acetophenone + *p*-dimethylaminoacetophenone	0.40	5.62 (5.52) 0.652 (0.654)	8.62—8.44
	0.53	5.58 (5.47) 0.674 (0.664)	8.28—8.24
	0.745	5.61 (5.56) 0.659 (0.658)	8.51—8.45
(ii) Acetophenone + *m*-nitroacetophenone	0.48	5.42 (5.30) 22.5 (25.5)	4.15—4.81
	0.785	5.57 (5.57) 24.9 (25.0)	4.47—4.49
	0.30	5.65 (5.72) 23.7 (24.8)	4.19—4.34
(iii) *m*-Nitroacetophenone + *p*-bromoacetophenone	0.56	(24.2) (8.89)	2.72
	0.80	(23.9) (8.94)	2.65
	0.30	(23.9) (9.01)	2.65

[a] The fraction x_A refers to the more reactive site. [b] The computer-generated rate constants are given in parentheses.

Table 2. Rate constants $k^T_B/10^{-3}$ dm^3 mol^{-1} s^{-1} for the detritiation and deprotonation of methyl neopentyl ketone.

Temp/°C	Solvent system	$10^3 k^T_B$ methyl	$10^3 k^T_B$ methylene	Rate ratio	Ref.
25.0	OH^-–H_2O	2.24	0.914	2.45	10(*b*)
25.0	OH^-–H_2O	2.23	0.0863	25.8	present work
25.0	OMe^-–MeOH	8.44	0.242	34.9	present work
25.0	OEt^-–EtOH	41.2	0.747	55.1	present work
32.0	OD^- in dioxane–D_2O (2:1)	53.2[a]	2.77[a]	19.2	2

[a] Refers to deprotonation.



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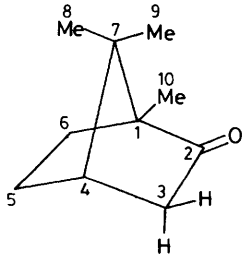

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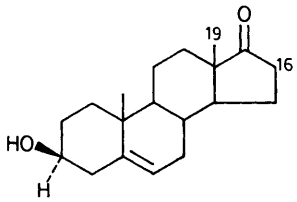

(3)

relatively high rate constant ratios, both the graphical and computer-based methods for obtaining the individual rate constants work well and the derived values are in good agreement with those reported in the literature. However below rate ratios of *ca.* 4:1 only the computer-based procedure was found to operate satisfactorily and here again good agreement with the literature values[19] ($k^T_{OH^-}$ = 9.10 × 10^{-3} for *p*-Br and 23.7 × 10^{-3} dm^3 mol^{-1} s^{-1} for *m*-NO_2) was obtained (Table 1).

The results for methyl neopentyl ketone (Table 2) show that the rate ratio determined in this work is similar to the results of a deprotonation study in a mixed aqueous solvent[2] but some ten times higher than in another report.[10b] As a further check, methyl neopentyl ketone was specifically tritiated in the acetyl position and the derived rate constant (2.12 × 10^{-3} dm^3 mol^{-1} s^{-1}) was in good agreement with the value obtained from the twin-site exchange study. This particular compound is a good model for investigating the importance of steric factors; the results in Table 2 show that the rate ratio more than doubles as the base is changed, $OH^- \longrightarrow OMe^- \longrightarrow OEt^-$, in accord with the methylene hydrogen becoming relatively more difficult to abstract as the size of the base increases. A plot of the Taft parameter E_S *vs.* the logarithm of the exchange rate in aqueous media for $RCOCH_3$ (R = Me, Et, Pr^i, Bu^t) is linear (correlation coefficient 0.98), further indicating the importance of steric control.

The rate constants for the detritiation of [3-^{3}H]camphor at 70 °C gave an apparent selectivity ratio of 2.0 for *exo* to *endo* detritiation. The present data are compared in Table 3 with those reported by Lajunen and Pibacka[11] and it is clear that they find a slightly larger rate ratio than that determined here. However, the k_{exo}/k_{endo} ratios in that work vary non-uniformly with temperature and an Arrhenius extrapolation of their results (k^{70}_{exo} = 1.12 × 10^{-3} dm^3 mol^{-1} s^{-1}; k^{70}_{endo} = 4.18 × 10^{-4} dm^3 mol^{-1} s^{-1}) shows that the discrepancy is mainly due to differing *exo* rates. The value k_{exo}/k_{endo} = 2.0 at 70 °C can be compared with the 'direct exchange' ratio (H ⟶ D) of

Table 3. Detritiation rate constants (k_{exo}, $k_{endo}/10^{-4}$ dm^3 mol^{-1} s^{-1}) for [3-^{3}H]camphor in aqueous sodium hydroxide solutions.

T/°C	10^4k_{exo}	10^4k_{endo}	Rate ratio	Ref.
55.0	3.45	1.04	3.3	11
65.0	6.94	2.84	2.4	11
70.0	8.40	4.20	2.0	present work
75.0	15.0	6.26	2.4	11
85.0	36.9	13.6	2.7	11
	32.0	15.0	2.1	present work

ca. 20 at 25 °C,[21,22] and is of the same order of magnitude as k^D_{exo}/k^D_{endo} for dedeuteriation[24] (2.0), though the latter values refer to mixed solvent systems. Overall, there is reasonable agreement between the present results for the k_{exo}/k_{endo} ratio and literature values.

No curvature was observed in the graphical method for the detritiation of the steroid (**3**) and the optimisation program gave two rate constants that differed by $<1\%$. In this case we can conclude that there is no difference in the kinetic acidities of the C-16 hydrogens ($k^T_{OH^-}$ at 25 °C = 1.51 × 10^{-3} dm^3 mol^{-1} s^{-1}). Even in the presence of the much bulkier t-butoxide base ($k^T_{Bu^tO^-}$ = 0.235 dm^3 mol^{-1} s^{-1}) the situation is unchanged. It appears, therefore, that despite the presence of the C(19) angular methyl group, the C(16) protons do not experience steric differentiation towards abstraction by the bases investigated.

The establishment of the above procedure has ramifications in several areas, the most important of which concerns the current practice of evaluating transition state structure in proton transfer processes *via* the determination of Brönsted coefficients and primary hydrogen isotope effects. An alternative strategy now emerges in which such parameters can be determined for two sites within the same compound and analysed in terms of structural and stereoelectronic considerations.[20–27] Furthermore, the results could also yield information on factors thought to be responsible for the differing reactivities of diastereotopic protons in compounds such as ketones and sulphoxides *e.g.* steric phenomena such as angle strain, non-bonded repulsion and torsional strain and electronic phenomena such as inductive and hybridisation effects, homoenolic and homoallylic stabilisation.

Experimental

Materials.—Dry methanol and t-butyl alcohol were obtained by refluxing the alcohols in the presence of magnesium turnings and iodine for 3 h followed by fractional distillation. Stock sodium methoxide and t-butoxide solutions were prepared by adding freshly cut sodium metal to the respective alcohols under nitrogen in a dry box and stored in a desiccator.

Tritiation Procedure.—Typically[20] the substrate (30–200 mg) was dissolved in dioxane (0.5–1.5 cm^3), a pellet of NaOH was added, followed by tritiated water (5–10 mm^3, 50 Ci cm^{-3}), and the mixture was kept in a sealed tube for up to 48 h, usually at room temperature. The solution was then poured into water and the substrate extracted with a small quantity (10 cm^3) of chloroform before drying over anhydrous Na_2SO_4. At this stage the usual procedure was to remove the solvent by passing N_2 through the mixture, redissolve a fraction of the product in a deuteriated solvent such as $CDCl_3$ or CD_3SOCD_3, add an internal standard (Me_4Si) and finally obtain both the ^{1}H and ^{3}H NMR spectra. Diluted samples were also subjected to radiogas chromatographic analysis.

Tritiation of camphor by the above method gave a product

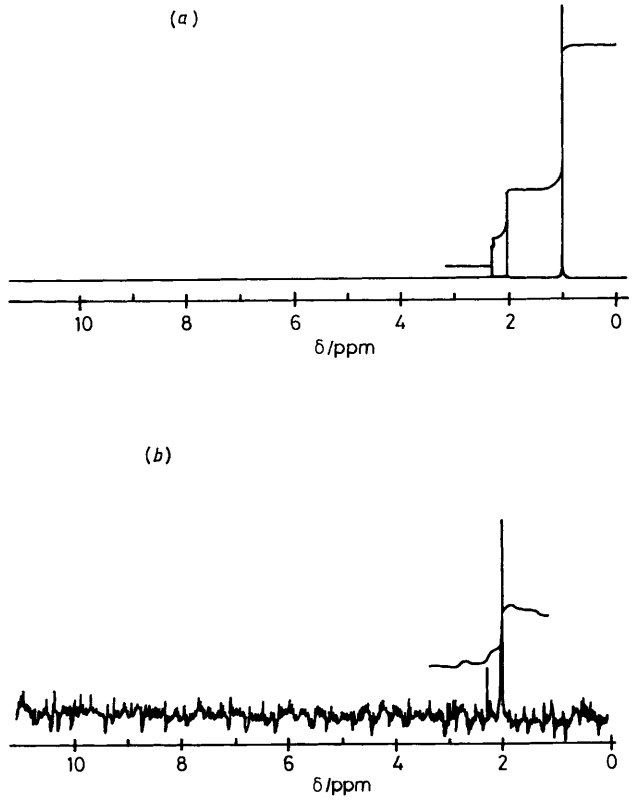

Figure 1. NMR spectra of methyl neopentyl ketone (**1**) labelled at the acetyl and methylene positions by exchange in HTO–OH$^-$ solutions: (*a*) ^{1}H spectrum, (*b*) ^{3}H spectrum (^{1}H decoupled).

the radioactivity of which (2 mCi g^{-1}) was sufficient for kinetic studies but provided no ^{3}H NMR spectrum. By contrast, use of dimethyl sulphoxide containing Me_4NOH proved to be both convenient and effective. Here, camphor (0.25 g) was dissolved in 1.0 cm^3 of DMSO, freshly dried ($CaSO_4$). To this solution was added HTO (5 mm^3, 250 mCi) and 25% Me_4NOH solution (4 mm^3). Sampling of the reaction mixture, after it had stood for 5 min at room temperature and then after 10 min intervals, showed that complete equilibration occurred within minutes after mixing. Quenching of the DMSO solution after 45 min by addition to dilute HCl (50 cm^3; 0.1 mol dm^{-3}), followed by extraction with pentane (2 × 20 cm^3), drying ($MgSO_4$) and rotary evaporation, yielded the desired labelled camphor (140 mCi) the purity of which was established by TLC.

Methyl neopentyl ketone specifically labelled in the acetyl group was prepared by tritiating the kinetic enolate of the ketone, formed by abstraction of the least hindered proton by the strongly hindered base, lithium di-isopropylamide. This was formed by adding to di-isopropylamine (4 × 10^{-3} mol dm^{-3}) an equimolar quantity of n-butyl-lithium in hexane and tetrahydrofuran (THF) (5 cm^3) at dry ice–acetone temperature under an atmosphere of nitrogen. After being stirred for 0.5 h, a solution of methyl neopentyl ketone (3.5 × 10^{-3} mol dm^{-3}) in THF (6 cm^3) was added over the course of 0.7 h and stirring was continued for a further 0.5 h. At this stage tritiated water (10 mm^3, 50 Ci ml^{-1}) and distilled water (70 mm^3) in THF (6 cm^3) were added with stirring over the course of 0.5 h, at the end of which time the dry ice–acetone bath was removed and the temperature allowed to rise. The solvent and amine were removed using a rotary evaporator, the ketone was extracted into ether (30 cm^3), the solution was washed with dilute HCl (10 cm^3) and water (5 cm^3) before being dried over anhydrous Na_2SO_4. Removal of the ether gave a product having a specific activity of (72 mCi cm^{-3}).

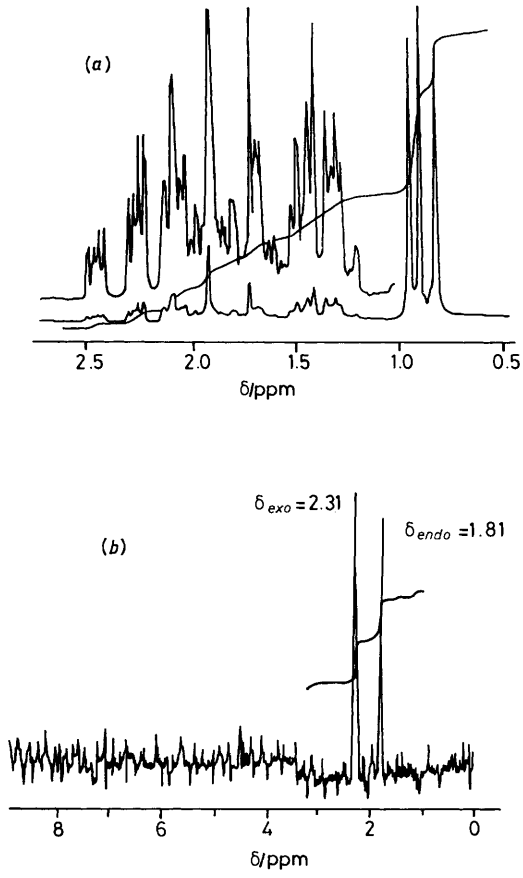

Figure 2. NMR spectra of camphor (**2**) labelled at the C(3) position: (*a*) ^1H, (*b*) ^3H (^1H decoupled).

^3H *NMR Analysis.*—For methyl neopentyl ketone (**1**) labelled by exchange in HTO–OH^- solutions, the ^3H (^1H decoupled) NMR spectrum is shown in Figure 1(*b*) with the ^1H NMR spectrum given in Figure 1(*a*) for comparison. The percentage relative tritium incorporation obtained by ^3N NMR integration is 76.9% at the methyl position and 23.1% at the methylene position. The ^3H NMR spectrum (not shown) of the compound prepared using lithium di-isopropylamide as the base indicated that at least 95% of the tritium was at the terminal methyl position.

The ^1H NMR spectrum of camphor [Figure 2(*a*)] is complex. The set of eight peaks centred around δ 2.3 integrate to one proton and can be assigned to the 3-*exo*-hydrogen since they give the expected pattern of splitting although the individual peak intensities are not first order. Coupling occurs with the 3-*endo*-hydrogen (J = 18 Hz), the bridgehead hydrogen at C-4 (J = 4.5 Hz) and there is a long range interaction (J *ca.* 2.5 Hz), probably arising from 'W' coupling with the 5-*exo*-proton.[28] Gradual disappearance of the δ 2.3 multiplet occurs when camphor is progressively deuteriated in dioxane containing $NaOD/D_2O$. The remainder of the spectrum in the region δ 1.0–2.0 is difficult to assign due to the overlapping of signals and extensive coupling within the system. However, the ^3H NMR spectrum of tritiated camphor [Figure 2(*b*)] is simple and leads to the unambiguous conclusion that the 'hidden' 3-*endo* signal is at δ 1.81.

For dehydroepiandrosterone (**3**) labelled at the C(16) position, the ^1H and ^3H (^1H decoupled) NMR spectra are shown in Figures 3(*a*) and 3(*b*), respectively. From integration of the ^3H spectrum, the tritium distribution between the C(16) labile positions (δ 1.98 and 2.38) appears to be equal.

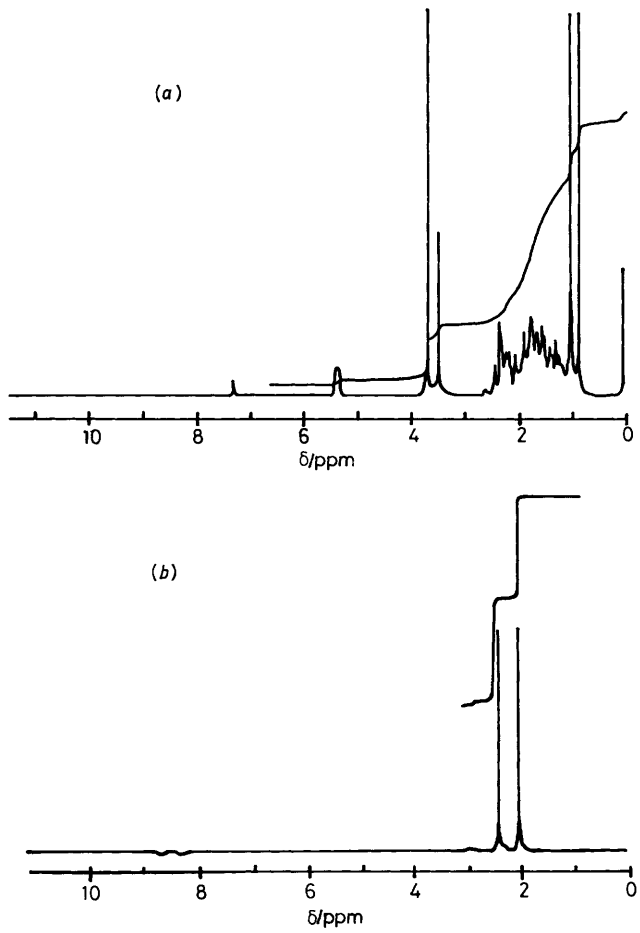

Figure 3. NMR spectra of steroid (**3**) labelled at the C(16) position: (*a*) ^1H, (*b*) ^3H (^1H decoupled).

Radio-gas Chromatographic Analysis.—This was carried out on stock solutions of labelled substrates in dioxane, using a modified Carlo–Erba Fractovap 4200 instrument fitted with a 5% OV1 on chromosorb G column and microprocessor controlled temperature programming as described previously.[17]

Kinetic Measurements.—The detritiations were carried out as described previously, with the difference that instead of the customary 10–12 samples, generally about 30 were analysed. This was necessary because of the curve-fitting nature of the experiment. Every sample count N_t leads to the calculation of a detritiation parameter, P_{obs}, which incorporates values of the initial and final radioactivities, N_0 and N_∞. Consequently a greater number of points was taken in the initial stages of the reaction so that an accurate value of N_0 could be obtained; usually N_∞ was very small. The reactions were generally followed over 90% completion.

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