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Review Article

Mechanistic Use of Short-Lived Radionuclides in Organic and Bio-Organic Chemistry

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Dedicated to Professor Göran Bergson on the occasion of his 65th birthday

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The use of the short-lived radionuclides ^{11}C and ^{18}F in the study of reaction mechanisms is described.

1. Introduction

The isotopes of elements are utilized in different ways in the investigation of reaction mechanisms: (i) as *tracers* and (ii) by determining *kinetic isotope effects*, i.e. effects on reaction rate caused by isotopic substitution.

Soon after the discovery of the hydrogen isotope deuterium, it was realized that isotopic substitution in a molecule might alter the rate of a chemical reaction and this phenomenon was indeed observed. However, it was not until the pioneering work by Bigeleisen, Westheimer and Melander at the end of the 1940s that determination of kinetic isotope effects (KIEs) was used as a method to obtain mechanistic information. Ever since then, determination of the effects of isotopic substitution on reaction rates and equilibria in chemical systems has been one of the most powerful tools for the elucidation of organic[1] and biochemical[2] reaction mechanisms. The theoretical basis for the interpretation of isotope effects was laid by Bigeleisen and Goeppert-Mayer[3] in a famous article published in 1947; the 50th anniversary of the so-called Bigeleisen equation was recently celebrated.[4]

The study of kinetic isotope effects yields answers to two fundamental mechanistic questions. (i) Which atoms undergo rate-limiting bonding change (forming or breaking of bonds)? (ii) What is the structure of the activated complex? Is the TS constant or does it vary when the system is perturbed by, say substitution? Is the TS reactant-like or product-like, or is it 'symmetric'? Do bonding changes take place synchronously or not?

There has been a tremendous development in the field. Applications today range from simple gas phase reactions[5] to complex enzyme reactions,[2] and a large number of organic reactions have been studied by means of KIEs.

Great progress has been made regarding experimental techniques as well as theoretical understanding and methodology; even the small but significant effects of heavy elements may be determined with high precision with the aid of mass spectrometry,[6] NMR[7] spectroscopy and radioactivity measurements.[6] Clever kinetic methods have been devised to minimize experimental errors.[8] It has become possible to calculate transition structures by quantum chemical methods, thus permitting isotope effects for different positions in a reacting system to be calculated.[9] Expressions such as 'experimental transition states'[9a] and 'isotope effect mapping of transition states' are found in the literature. A recently developed NMR method[7] allows several isotopic positions to be simultaneously studied and the use of this method has contributed to the mechanistic understanding of important synthetic organic reactions, e.g., the Sharpless osmohydroxylation.[10]

A new line of development which will be addressed in this paper is the use of accelerator-produced short-lived radionuclides in the study of isotope effects on reaction rate. Short-lived radionuclides today are largely used in biomedical research and clinical diagnosis in connection with positron emission tomography (PET).[11] The nuclides utilized include ^{11}C ($t_{1/2}=20.4$ min), ^{18}F ($t_{1/2}=110$ min), and ^{76}Br ($t_{1/2}=16$ h).

2. Fluorine kinetic isotope effects

Organofluorine chemistry is a very active and rapidly growing field.[12] In mechanistic investigations fluoride is

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commonly employed e.g. as a leaving group in elimination and substitution reactions. It therefore seemed worthwhile to try to include fluorine among the heavy atoms used for kinetic isotope effect measurements. Since natural fluorine consists of 100% ^{19}F and since no long-lived radioisotopes are available, the only way to accomplish determination of F KIEs is to use a short-lived radionuclide. Among these the accelerator-produced ^{18}F has a convenient half-life of 110 min and is routinely produced in many laboratories; the isotope ^{18}F is used in the labelling of radiopharmaceuticals and other compounds used for biomedical research and clinical diagnosis utilizing the PET-imaging technique.[11] Nucleophilic as well as electrophilic labelling reagents are available and quite a number of compounds have been labelled with ^{18}F; these include carbohydrates, alkyl halides, fatty acids and steroids.[13]

3. Carbon kinetic isotope effects

The advantage of using the short-lived carbon isotope ^{11}C in combination with the long-lived radioisotope ^{14}C is that the observed isotope effect is then maximised as compared with the ordinarily used $^{12}C/^{13}C$ or $^{12}C/^{14}C$ KIEs. Heavy element isotope effects like those for carbon are small so it is very valuable to increase the mass ratio in order to determine the KIEs with the highest possible precision. This is particularly important when studying the often small changes in KIE caused by system variations such as change of substituent, different steric requirements or choice of solvent.

4. Synthesis with short-lived radionuclides

Most traditional organic chemistry methods can be adapted to the synthesis of radiolabelled compounds. However, modifications of the original synthetic method are often needed for application to labelling chemistry and sometimes new synthetic strategies must be designed. Normally the synthetic route is chosen so that only a few rapid steps remain when the radionuclide has been incorporated. Synthesis of tracers labelled with short-lived radionuclides involves production of the radionuclide and the labelling precursor, synthesis of the tracer molecule, purification and analysis.

During the synthesis of compounds labelled with short-lived radionuclides, the most important parameter is time. While the build-up of the product is governed by the kinetics of the chemical transformation, there is always the competing decay of the radionuclide. Consequently the optimal reaction time with regard to radiochemical yield is determined by the product of these two parameters.[14]

Labelling with ^{11}C. The most commonly used starting material in ^{11}C synthesis is $[^{11}C]CO_2$.[15] It is produced by bombardment of nitrogen gas with high energy protons, the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. Trace amounts of oxygen gas in the target gas combine with the high energy ^{11}C atoms to produce $[^{11}C]CO_2$ as a primary precursor.[15] Since the number of chemical transformations that can be achieved with $[^{11}C]CO_2$ is limited the primary precursor is generally converted into a more reactive secondary precursor which can be further used to label a molecule of interest. Some of the secondary precursors available from $[^{11}C]CO_2$ are summarised in Fig. 1.

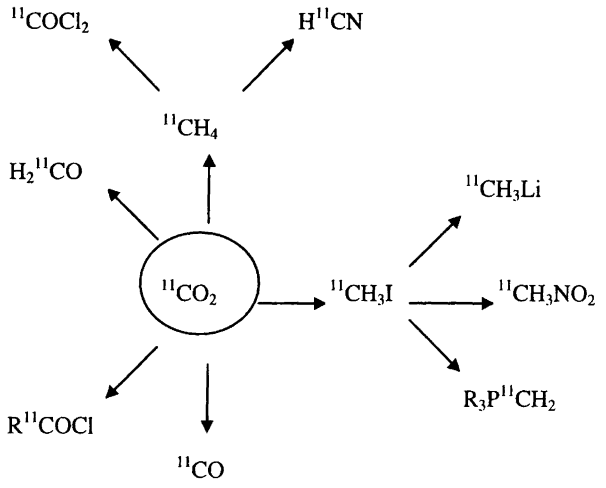


Fig. 1. Some useful ^{11}C labelled secondary precursors obtained from [^{11}C]carbon dioxide.

Labelling with ^{18}F. Introduction of ^{18}F into a molecule can be achieved by electrophilic or nucleophilic addition of the radionuclide.[13] Common methods for ^{18}F preparation include the $^{20}Ne(d,\alpha)^{18}F$ and $^{18}O(p,n)^{18}F$ nuclear reactions. Both methods can give either $[^{18}F]F_2$ or $[^{18}F]F^-$.

Electrophilic fluorination with $[^{18}F]F_2$ provides a facile means of introducing ^{18}F into electron-rich compounds such as alkenes or aromatic rings. Owing to its rather unspecific reactivity the use of electrophilic fluorine is limited. Improvements have been achieved by deactivating the fluorine by dilution with an inert gas or formation of new fluorinating agents, $[^{18}F]CH_3COOF$ being the most useful.[13,16]

Nucleophilic fluorination with $[^{18}F]F^-$ is usually accomplished via two main approaches: (a) nucleophilic displacement of a halide or a sulfonate group in unhindered aliphatic systems, and (b) nucleophilic aromatic substitution of a nitro or trialkylammonium group in activated aromatic systems. Since the nucleophilicity of the fluoride ion is low in aqueous and protic solvents due to strong solvation, reactions are normally performed in aprotic solvents. Phase transfer reagents such as quaternary ammonium salts or a potassium counterion complexed by macrocyclic ethers or the kryptand Kryptofix 2.2.2 are used to activate and increase the solubility of the fluoride.[13,15]

5. $^{11}C/^{14}C$ and $^{18}F/^{19}F$ kinetic methods – a combination of liquid chromatography and liquid scintillation

The methodology for determining KIEs using short-lived radionuclides is based on separation of reactants and products by liquid chromatography followed by radioactivity measurements using liquid scintillation. The method for determination of carbon KIEs has certain advantages except for the already mentioned fact that the largest practical mass ratio of carbon isotopes is used: (i) the HPLC technique is usually easily applied to different chemical systems; (ii) no work-up or degradation of the samples is required; (iii) the analyses are insensitive to unlabelled impurities; (iv) both isotopic species can be quantitatively determined with high precision using the same instrument (v) the large difference in half-life for the two carbon isotopes used simplifies the measurement.

Experiments with ^{11}C and ^{18}F imply rapid execution and the experiments have to be carefully prepared before the synthesis is started. The different steps in the $^{11}C/^{14}C$ KIE method are:[17] (1) synthesis and purification of the ^{14}C-labelled substrate; (2) synthesis and purification of the ^{11}C-labelled substrate; (3) initiation of the kinetic experiment by mixing of the isotopically labelled substrates with the other reagents of the reaction; (4) sampling and quenching of the reaction at suitable time intervals; (5) HPLC separation and collection of the fractions with the labelled substrate and product in scintillation bottles; (6) measurements of the total ($^{11}C + ^{14}C$) radioactivity followed, after decay of the ^{11}C, by measurement of ^{14}C-radioactivity of the fractions, half-life correction of ^{11}C-data; (7) calculation of the KIE.

For the $^{18}F/^{19}F$ KIE method[18] the order of the different steps is the same, exchanging the ^{11}C for ^{18}F and ^{14}C for ^{19}F. The unlabelled, i.e. ^{19}F-substrate, is quantitatively detected by means of the HPLC UV detector.

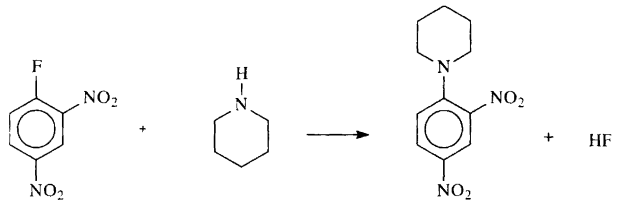
6. Determination of rate limiting steps using leaving group F KIEs – the case of nucleophilic aromatic substitution

Leaving group KIEs are fairly easy to interpret and have been utilized for a long time in mechanistic investigations of nucleophilic aliphatic substitution[19] and elimination reactions.[20] A leaving group KIE is expected to increase monotonously with increasing degree of bond breaking between the isotopic leaving group atom and the α-carbon in the transition state of the rate limiting step. Thus sulfur, oxygen, and chlorine leaving group KIEs have been reported.[19] Fluorine has also been employed as the leaving group in many other mechanistic studies of this reaction type.

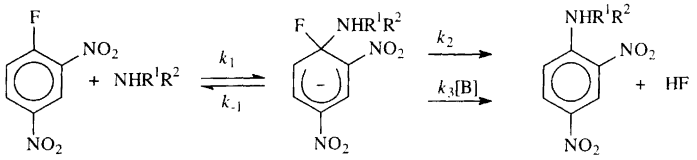
Displacement reactions on activated aromatic molecules have been the subject of considerable mechanistic interest over the years. A great deal of attention has been directed to nitroaromatics with halogen or other leaving groups.[21] The influence of nucleophile, solvent, leaving group, and the presence or absence of base catalysis on kinetic parameters are some of the system variations that have been employed in these mechanistic investigations.

The generally accepted mechanism for nucleophilic aromatic substitution of activated substrates (the S_NAr mechanism) is an addition–elimination and involves the formation of a Meisenheimer type of intermediate.[21] Whether the rate limiting step of this mechanism is the formation of the intermediate or expulsion of the leaving group has been found to depend on the character of the nucleophile and the leaving group as well as on the solvent. Decomposition of the intermediate may be base catalysed as indicated in Scheme 1 ($k_3[B]$). Either the nucleophile or some of the added base acts as the catalyst. The observation of base catalysis has been used as a mechanistic criterion of whether the formation or the decomposition of the intermediate is rate limiting.

The nucleophilic substitution of 2,4-dinitrofluorobenzene (DNFB) with secondary amines is a good model system for the demonstration of a fluorine KIE. The existence of a significant leaving group F KIE of 1.0262(7) for the reaction of DNFB with piperidine in tetrahydrofuran (THF) at 30 °C (Scheme 2), unequivocally demonstrates that C–F bond cleavage is rate limiting in that system.[22] The value is of the same order of magnitude as might be expected from an estimate of the maximal $^{18}F/^{19}F$ KIE for complete loss of zero-point energy for a simple two-centre model of a C–*F bond,



Scheme 2.



Scheme 1.

which yields a value of ca. 3% for a typical aromatic C–^{19}F stretching frequency of 1250 cm^{-1}.[22]

6.1. The effect of solvent on the rate limiting step. The leaving group F KIE probe offers an opportunity to learn whether the rate limiting step is affected by change of solvent. On the basis of an investigation of base catalysis, Nudelman has earlier concluded that a shift from rate limiting elimination to rate limiting addition takes place when the solvent is changed to one with slightly hydrogen-bond accepting properties, e.g. acetonitrile, from one lacking such properties, e.g. THF.[23] The resulting isotope effect observed upon change of solvent is interesting and permits conclusions regarding the rate limiting step to be drawn; it also further proves that the observed KIE is real and not an artifact.

The isotopic rate constant ratio obtained from the kinetic experiments in acetonitrile was 0.9982(4).[18] Thus, in acetonitrile no significant fluorine KIE is observed, although the small inverse value determined might be attributed to the very small secondary effect expected for rate limiting formation of the intermediate involving sp^3 to sp^2 rehybridization.

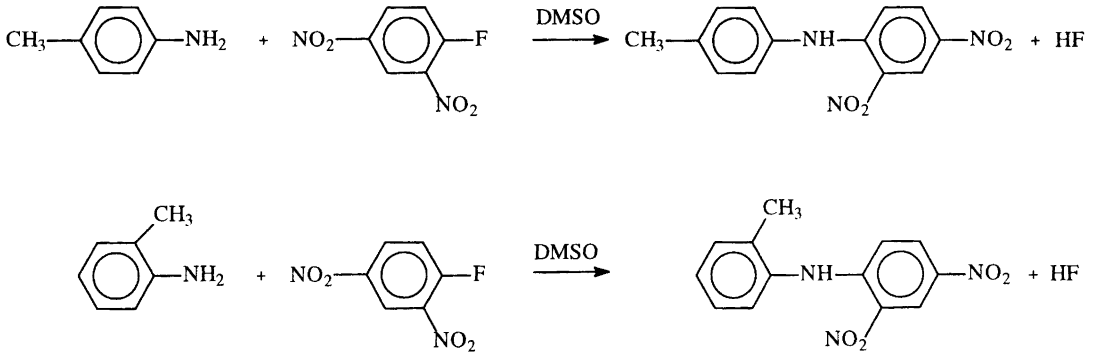
6.2. The effect of steric hindrance on the rate limiting step. Much mechanistic information is available concerning electronic effects, whereas, the influence of steric effects on the mechanism is less well investigated. However, Onyido and Hirst have employed 2-methylaniline and 4-methylaniline as nucleophiles in the reaction with 2,4-dinitrofluorobenzene (DNFB) in DMSO[24] (Scheme 3). From studies of base catalysis they concluded that a change in the rate limiting step was induced by the steric effect of the *o*-methyl group. The rate of reaction was reduced by a factor of 198 when the position of the methyl substituent was changed from *para* to *ortho* in the nucleophile. A change in the rate-limiting step induced by changing the steric properties of the nucleophile should be confirmed by determination of the F KIEs.

The KIE determined in DMSO at 30 °C was 1.0005(30) for 4-methylaniline and 1.0119(37) for 2-methylaniline.[25] The significant F KIE observed for the reaction between DNFB and the sterically more hindered nucleophile 2-methylaniline shows that expulsion of the nucleofuge is at least partially rate-limiting. The F KIE for the reaction of 4-methylaniline is virtually nil and is thus consistent with rate-limiting addition of the nucleophile to the substrate.

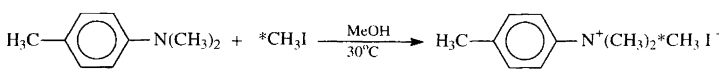
7. Transition state structure – the case of nucleophilic aliphatic substitution

7.1. Relative carbon KIEs. The first ^{11}C/^{14}C KIE reported was the primary carbon isotope effect for the methylation of *N,N*-dimethyl-*p*-toluidine with methyl iodide,[26,17] Scheme 4. One of the reasons for choosing this reaction in that methodological study was that a fairly large ^{12}C/^{14}C KIE of 1.117(11), determined in methanol at 48.5 °C, had been reported by Buist and Bender.[27] At 30 °C the ^{11}C/^{14}C KIE was found to be 1.202(8), which is close to the estimated maximal value. In another study the hydroxide ion was used as nucleophile. In 50% dioxane–water at 25 °C the ^{11}C/^{14}C KIE was determined to 1.192±0.001.[28] As exemplified by these two S_N2 reactions of methyl iodide, where different nucleophiles have been used, the primary carbon KIE is large, which demonstrates that the method can undoubtedly be used as a tool for obtaining mechanistic information.

Carbon isotope effects in the reaction of hydroxide ion with methyl iodide have earlier been studied by Bender and Hoeg[29] who determined the ^{12}C/^{14}C KIE to be 1.088(10) in 50% dioxane–water at 25 °C, and by Lynn and Yankwich[30] who determined the ^{12}C/^{13}C KIE to be 1.035(6) in water at 31 °C. The latter value is smaller than expected from the ^{12}C/^{14}C KIE value using a simple



Scheme 3.



Scheme 4.

theoretical model, presented by Bigeleisen,[31] according to which the relative strengths of $^{12}C/^{13}C$ and $^{12}C/^{14}C$ KIEs are expressed by eqn. (1).

$$r = \ln(k_{12}/k_{14})/\ln(k_{12}/k_{13}) \approx 1.9 \quad (1)$$

It has been suggested that this deviation was caused by experimental errors or due to the fact that the KIEs were determined in different solvents.[32] A corresponding relationship between $^{11}C/^{14}C$ and $^{12}C/^{14}C$ KIEs can be derived yielding the value of $r \approx 1.6$. From the experimentally determined $^{11}C/^{14}C$ KIE, the $^{12}C/^{14}C$ KIE can thus be predicted to be 1.116. Again the experimental value (1.088) is smaller than that predicted from the theoretical model. Most experimental data reported in the literature have been shown to conform with eqn. (1),[33] but this is obviously not the case for the reaction between hydroxide ion and methyl iodide.

Application of the relationship above to the reaction of *N,N*-dimethyl-*p*-toluidine with methyl iodide, yields a predicted $^{12}C/^{14}C$ KIE of 1.122 calculated from the experimentally determined $^{11}C/^{14}C$ value. This is, to within experimental error, equal to the KIE observed by Buist and Bender (1.117).

7.2. Labelled central atom. Probing steric effects. Steric effects are of great importance in controlling reactivity and selectivity of chemical reactions. Systematic experimental and theoretical investigations are, however, much more scarce when it comes to steric effects than they are for electronic (substituent) effects. The quaternization reaction of tertiary amines by alkyl substrates (the Menshutkin reaction) has been extensively studied in solution[34] with respect to effects of variations in substituents, leaving group, solvent, etc., and reactivity–selectivity relationships have been considered as indicators of structural variations of the transition state.

The Menshutkin reaction using pyridines as the nucleophile is strongly affected by the size of substituents in the *ortho* positions and is one for which steric effects have been systematically studied, and for which steric and electronic effects can be separated.[35] Kinetic isotope effect techniques have also been applied to the Menshutkin reaction with respect to steric effects. Thus primary $^{35}Cl/^{37}Cl$ leaving group KIEs were determined for the quaternizations of triethylamine and quinuclidine[36] and of pyridine and 2,6-lutidine[37] by methyl chloride. Such KIEs are, in principle, easily interpreted since they decrease monotonically with the magnitude of the stretching force constant for C–X within the TS. However, these KIEs are very small and difficult to measure accurately, and in the cases mentioned show opposite results.

Inverse deuterium secondary KIEs are observed for 2-alkyl- and 2,6-dialkyl-pyridines in their reactions with methyl iodide.[38,39] The inverse KIE was attributed by Brown and McDonald[8] to the smaller size of CD_3 than CH_3, but Balaban and coworkers[9] concluded that both steric and electronic contributions were involved. In contrast, the KIEs are almost nil for 3- and 4-substituted (CH_3 and CD_3) pyridines.

One KIE probe which might yield information on TS variation due to steric perturbation is the primary central carbon KIE. These KIEs were determined for the quaternization reactions of pairs of tertiary amines, which exhibit essentially the same electronic contribution (measured as pK_a of the corresponding ammonium ion). Thus variations in reaction rate are sterically induced.

The resulting KIEs determined in acetonitrile at 30.00 °C are 1.220(9) and 1.189(12) for 2,6-lutidine and 2,4-lutidine, respectively.[40] There are two main features in the observed data.

(i) The observed primary $^{11}C/^{14}C$ KIEs are large for both reactions, close to maximal as estimated on the basis of loss of zero point energy on going from initial to transition state. A large primary carbon kinetic isotope effect of $k^{11}/k^{14} = 1.202(8)$ has previously been determined for the reaction of *N,N*-dimethyl-*p*-toluidine with methyl iodide in methanol at 30 °C.[17] As for the case of deuterium KIEs on hydron transfer, maximal primary carbon KIEs are expected for 'symmetric' transition states where donor and acceptor are bound with equal strength to the isotopic atom being transferred. Some cases of variation in the strength of the carbon KIE with reactivity have been observed experimentally[41] and theoretical calculations point in the same direction.

(ii) A small increase in primary carbon KIE is observed for 2,6-lutidine as compared with 2,4-lutidine. Thus steric hindrance seems to be reflected in a higher primary central carbon KIE for this reaction system since electronic effects are the same. Steric hindrance has often been associated with large primary *deuterium* KIEs, a fact which has usually been explained by a larger amount of tunnelling. One example of this is the primary deuterium KIE of 24.8 reported by Lewis and Funderburk for proton transfer to 2,4,6-trimethylpyridine.[42]

More information regarding the structural variation of the TS may be obtained if the present results are viewed within the context of previously published KIE data for other labelled positions in similar reaction systems. The combined evidence from incoming and leaving group KIEs for the reactions of the substituted pyridines thus seems to demonstrate a structural variation of the TS as the steric demand of the nucleophile changes. Steric hindrance as modelled by dimethyl substitution in the 2- and 6-positions to the nucleophilic nitrogen increases the C–X bond distance as reflected in the larger leaving group chlorine KIE. Thus the sterically more hindered nucleophile has a slightly looser TS which should be expected to yield a larger primary central carbon KIE. This is also what we observe experimentally.

7.3. Labelled nucleophiles. The effect of substitution in the substrate or in the leaving group. Isotope effects arising from labelling of the incoming group (nucleophile) of a substitution reaction are interesting since they would

Scheme 5.

provide knowledge concerning the amount of bond formation of the new bond in the transition state. Several workers have attempted to use such KIEs to determine the nucleophile–α-carbon bond formation in an S_N2 reaction. These attempts have been largely unsuccessful, however, because these KIEs were invariably very small. Therefore it was difficult to demonstrate that they were real and, in addition, the effects were too small to indicate how changes in the structure of the system affect the KIE and transition state structure. This is an ideal case to utilize the fact that the carbon KIEs are maximized by using $^{11}C/^{14}C$.

The S_N2 reactions between a series of *p*-substituted benzyl chlorides and carbon labelled cyanide ion, Scheme 5, were chosen as models to determine whether one could measure a significant (larger than experimental error) incoming group kinetic isotope effect, and whether these isotope effects could be used to model the S_N2 transition state.[43]

The bonding to the labelled carbon atom of the nucleophile will be greater in the TS than in the reactants because the nucleophile–α-carbon bond is formed in the S_N2 TS. As a result, the vibrational energy of the labelled carbon will be greater in the TS and the primary incoming group KIE will decrease with increased bond formation. The observed KIE has been estimated to be between 1.02 and 0.87 for transition states ranging from reactant- to product-like.[43] The actual value will, of course, be determined by the extent of nucleophile–α-carbon bonding in the S_N2 transition state.

The $^{11}C/^{14}C$ KIE found in the benzyl chloride–cyanide ion S_N2 reaction is large enough [1.0105(2), see Table 1][43] to suggest that these isotope effects can be used to learn how substituents on the benzene ring of the substrate affect the length of the nucleophile–α-carbon bond in the S_N2 transition state. The $^{11}C/^{14}C$ KIEs for the S_N2 reactions between a series of *p*-substituted benzyl chlorides and cyanide ion in 20% aqueous DMSO at 30 °C, Table 1, were measured to test this hypothesis.[43] The very small change in the magnitude of these incoming group kinetic isotope effects suggests that there is little or no change in the nucleophile–α-carbon bond in these S_N2 transition states when the *p*-substituent on the benzene ring is altered.

Table 1. Changing the substrate by substitution. The carbon incoming group[43] and chlorine leaving group[44] kinetic isotope effects for the S_N2 reactions between labelled cyanide ion and a series of *p*-substituted benzyl chlorides.

Para-substituent	k^{11}/k^{14} [a]	k^{35}/k^{37} [b]
CH_3	1.0104(10)	1.0079(4)
H	1.0105(20)	1.0072(3)
Cl	1.0070(10)	1.0060(2)
NO_2	—	1.0057(2)

[a] Measured at 30 °C in 20% (v/v) aqueous DMSO. [b] Measured at 20 °C in 20% (v/v) aqueous dioxane.

It would be desirable to be able to model the changes in these S_N2 transition states in more detail. This was possible because Hill and Fry[44] have measured the chlorine leaving group (k^{35}/k^{37}) kinetic isotope effects for the same S_N2 reactions (in 20% aqueous dioxane). The k^{35}/k^{37} isotope effects in Table 1 can be used to indicate how the α-carbon–leaving group (the $C_\alpha \cdots Cl$) transition state bond varies when the *para*-substituent is altered in these S_N2 reactions.

The smaller chlorine leaving group kinetic isotope effects found for the reactions with the more electron-withdrawing substituents clearly demonstrate that the α-carbon–leaving group transition state bond becomes shorter as a more electron-withdrawing substituent is added to the benzene ring of the substrate. Moreover, the change in the chlorine isotope effects with *para*-substituent is large, i.e., it is 14% of the theoretical maximum chlorine isotope effect. In comparison, the change in the incoming group carbon isotope effects is only 2.3% of the theoretical maximum. As a result, the isotope effect data suggest that addition of an electron-withdrawing substituent to the *para*-position of the benzene ring of the substrate causes little or no change in the nucleophile–α-carbon transition state bond but a significant shortening of the α-carbon–leaving group bond when a more electron withdrawing substituent is placed in the substrate, Fig. 2.

The incoming group KIE has also been used to determine how a change in substituent in the leaving group affects the structure of the S_N2 transition state. The system chosen for this investigation is the S_N2 reaction between a series of *m*-chlorobenzyl *p*-substituted benzenesulfonates with cyanide ion in 0.5% aqueous acetonitrile,[45] Scheme 6.

These substrates were chosen because (i) the *para* substituent on the leaving group could be changed easily and (ii) benzylic benzenesulfonates with an electron-withdrawing substituent on the benzene ring of the benzyl

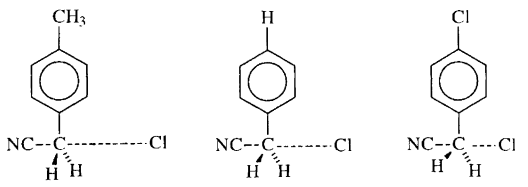
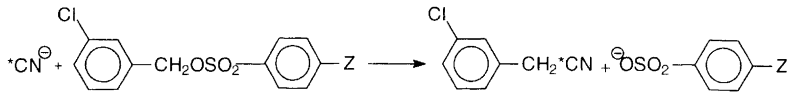


Fig. 2. The relative structures for the S_N2 transition states for the reactions of *para*-substituted benzyl chlorides with cyanide ion.



Scheme 6.

group are more stable than unsubstituted benzylic benzenesulfonates.

The primary incoming group $^{11}C/^{14}C$ KIEs, found when isotopically labelled cyanide ion was reacted with *m*-chlorobenzyl *p*-substituted benzenesulfonates, are presented in Table 2. The KIE decreases slightly as a more electron-withdrawing substituent is added to the leaving group, i.e., it decrease from 1.0119(10) for the *p*-methylbenzene sulfonate leaving group to 1.0096(5) for the *p*-chloro. However, the change in the isotope effect with the substituent is small. Since the magnitude of these isotope effects decreases as the amount of nucleophile–α-carbon bond formation increases in the transition state, these isotope effects suggest that the amount of cyanide–α-carbon bond formation increases slightly as a more electron-withdrawing group is added to the leaving group. The isotope effects found in the reaction between cyanide and the *m*-chlorobenzyl benzenesulfonates are slightly larger than those found for the benzyl chlorides indicating that there is less cyanide–α-carbon bond formation in the arenesulfonate S_N2 transition states.

7.4. The determination of secondary deuterium KIEs with the aid of radioactive carbon. Secondary $^1H/^2H$ KIEs have proved to be informative on the TS structure of many reactions including nucleophilic aliphatic substitutions.[8] Since a secondary deuterium isotope effect results from an isotopic substitution at a position where no bond formation or breaking occurs these KIEs are usually close to unity. As a consequence careful kinetic measurements are required to determine these effects with an acceptable degree of accuracy. The use of ^{11}C in combination with ^{14}C offers an interesting way to determine secondary deuterium KIEs with high precision. This has been demonstrated for the S_N2 reaction between methyl iodide and hydroxide ion, which was studied by means of a double labelling technique.[28] The α-carbon KIE was first determined by the $^{11}C/^{14}C$ method. The experiment was then repeated, but this time using a substrate mixture where either the ^{11}C- or the ^{14}C-labelled methyl iodide was doubly labelled with deuterium, i.e., the substrate was either $^{11}CH_3I$ and $^{14}CD_3I$ or $^{11}CD_3I$ and $^{14}CH_3I$, where D is ^2H. The notation for the different rate constants is given in Scheme 7.

The different α-carbon KIEs obtained in this way, for the reaction performed in 50% dioxane–water at 25 °C, were $k_{11}/k_{14}=1.192(1)$, $k_{11}/k_{14D}=1.051(6)$ and $k_{11D}/k_{14}=1.326(5)$. Assuming that the carbon and deuterium KIEs are multiplicative, i.e., that the rule of the geometric mean holds, the secondary deuterium KIEs could be calculated from these isotope effects. The deuterium-content-corrected secondary α-deuterium KIEs were thus found to be 0.881(12) and 0.896(11) when the doubly labelled substrates were $^{11}CD_3I$ and $^{14}CD_3I$, respectively.

The secondary isotope effects originate from changes in the force constants upon going from reactants to the TS of the rate-determining step of the reaction. For the most part, secondary isotope effects depend on the change in zero-point energy. Any isotopically sensitive vibrational frequency that decreases on going from reactant to transition state contributes to a normal isotope effect (KIE > 1). A corresponding increase in vibrational frequency decreases the KIE and may even yield an inverse effect (KIE < 1). Inverse secondary α-deuterium KIEs are often observed for reactions proceeding via the S_N2 mechanism.[8,46] Until recently, these small KIEs have been attributed to steric interference by the leaving group and/or the nucleophile with the C–H(D) out-of-plane bending vibrations of the trigonal bipyramidal S_N2 TS. However, this view has had to be modified in the light of recent results obtained from several different theoretical calculations, which have shown that the C–H(D) stretching vibration contribution to the isotope effect is more important than previously thought.[8]

Table 2. Changing the leaving group. The incoming group kinetic isotope effects for the S_N2 reactions between a series of *m*-chlorobenzyl *p*-substituted benzenesulfonates and labelled cyanide ions in 0.5% aqueous acetonitrile at 0 °C.[45]

Para-Substituent	k^{11}/k^{14}
CH_3	1.0119(10)[a]
H	1.0111(20)
Cl	1.0096(5)

[a]The error is the standard deviation of the isotope effects found in at least four different experiments.

8. Secondary carbon KIEs in proton transfer reactions

Only a few cases of secondary carbon KIEs, utilizing the isotopes ^{13}C and ^{14}C together with the normal carbon

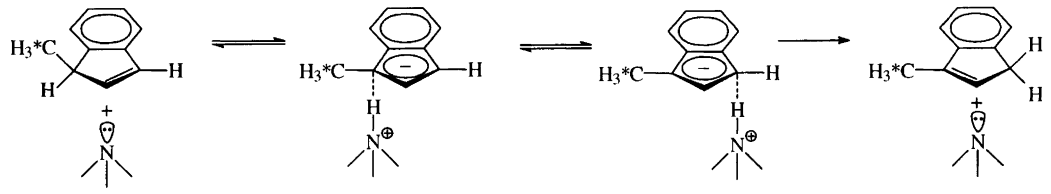
$$^{11}CH_3I + OH^- \xrightarrow{k_{11}} {}^{11}CH_3OH + I^-$$

$$^{14}CH_3I + OH^- \xrightarrow{k_{14}} {}^{14}CH_3OH + I^-$$

$$^{11}CD_3I + OH^- \xrightarrow{k_{11D}} {}^{11}CD_3OH + I^-$$

$$^{14}CD_3I + OH^- \xrightarrow{k_{14D}} {}^{14}CD_3OH + I^-$$

Scheme 7.



Scheme 8.

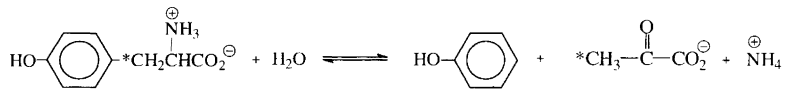
isotope, have been reported.[47] These results clearly indicate that the secondary carbon KIE, although small in magnitude, is significant. The potential value of its use as a probe of subtle mechanistic details is, of course, dependent on the possibilities of making accurate measurements. An approach to the solution of this problem is to make use of the full mass range of carbon isotopes, i.e., $^{11}C/^{14}C$.

Amine-catalysed 1,3-proton transfer in indene and its alkyl-substituted analogues is believed to occur via two or more hydrogen-bonded ion-pair intermediates. Both primary $^{1}H/^{2}H$ and secondary β-deuterium KIEs have earlier been determined for the reaction of 1-methylindene to 3-methylindene catalysed by DABCO and other tertiary amines.[48] The rearrangement is irreversible for this substrate and a plausible mechanism is shown in Scheme 8.

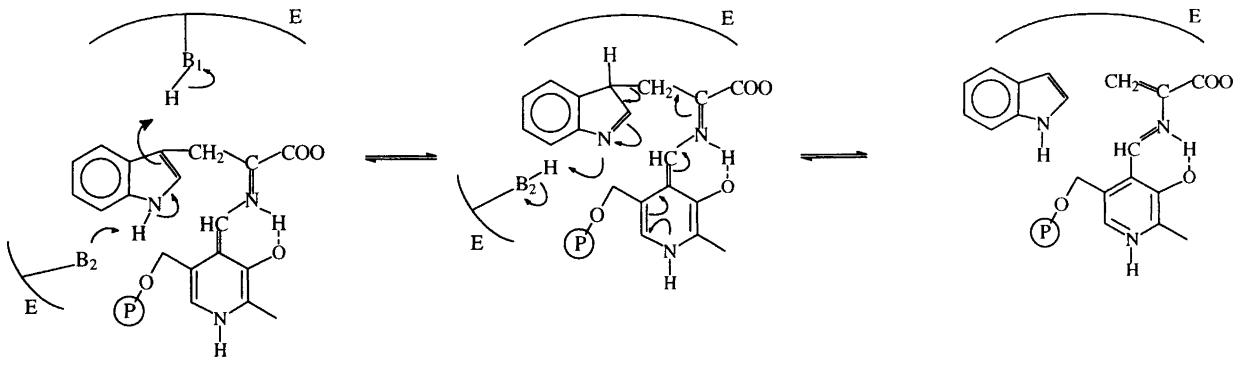
The first proton-abstraction step is at least partially rate determining as demonstrated by the observation of an experimentally primary $^{1}H/^{2}H$ KIE of 5.03 in toluene at 20 °C. The secondary carbon KIE (with the carbon label in the methyl group), in benzene at the same temperature, was found to be 1.010(5).[49] This $^{11}C/^{14}C$ isotope effect might have its origin in the hybridization change for which the largest vibrational frequency change is associated with the transformation of a valence angle bending mode to an out-of-plane bending mode in the limiting intermediate-like TS. The rehybridization causes a normal secondary KIE since the force constant for this mode is decreased.

Other effects that might contribute to the secondary isotope effect are hyperconjugative and/or inductive interactions between the negative charge on C-1 of the indene ring and the methyl group in the TS. A simple valence bond or MO picture shows that such anionic hyperconjugation causes bond lengthening and decreased force constants for the Ç–H bonds of the methyl group, since electronic charge is fed into the lowest vacant group orbital, which is antibonding. This interaction will be maximal when the C–C bond is perpendicular to the p-orbital on C-1. While the strength of the C–H bonds are diminished a simultaneous increase of C–C bonding is expected from the simple hyperconjugative picture. The exact balancing of these two factors contributing to the force field will affect the size and direction of the secondary carbon KIE.

Kinetic isotope effect techniques have proved to be powerful in establishing possible TS structures for a particular reaction especially when experimental methods and model calculations are combined. Upon comparing the experimentally determined values of the secondary $^{11}C/^{14}C$ isotope effect of 1.01[46] and the secondary β-$^{2}H_3$ effect (the deuterium in the methyl group) of 1.103[45a] with the results from model calculations, based on MNDO combined with BEBOVIB, they correspond to a bond order of around 0.7 for the forming N–H bond



Scheme 9. The tyrosine phenol-lyase catalysed reaction. The position of the label is indicated by an asterisk.



Scheme 10. The tautomerization and carbon–carbon bond breaking steps.

and a charge fraction of at least 0.3 localized on the carbon atom undergoing bond cleavage in the TS.[46] These results are also consistent with the Brønsted coefficient of 0.79 determined for this reaction, indicating a rather ion-pair intermediate-like TS. The results indicate that the methyl group carbon KIE seems to be a promising complement to the secondary β-deuterium KIE in probing anionic hyperconjugation.

9. Isotope effects in bio-organic catalysis – $^{11}C/^{14}C$-kinetic isotope effects in an enzyme-catalysed reaction

Kinetic isotope effects are well established tools also for the study of enzyme-catalysed reactions and yield information on kinetic as well as chemical mechanisms and on rate determining steps. There are three different methods for KIE measurements in enzyme reactions:[50] the direct comparison method, the equilibrium perturbation method, and the internal competition method.

The $^{11}C/^{14}C$ KIE method is an internal competition method, in which the different labelled substrates are mixed with the enzyme in the same reaction mixture. The isotope effects obtained by the $^{11}C/^{14}C$ method are ratios of the rates of the ^{11}C- and ^{14}C-reactions. For enzymes that follow the Michaelis–Menten equation[51] the velocity, v, is given by eqn. (2),

$$v = \frac{V[S]}{K + [S]} \tag{2}$$

where [S] is the substrate concentration, K is the Michaelis–Menten constant, and V is the maximum velocity.

The KIE from a competition experiment for an enzyme catalysed reaction is given by eqn. (3)

$$\text{KIE} = {}^{11}(V/K)/{}^{14}(V/K) \tag{3}$$

Tyrosine phenol-lyase catalysed α,β-elimination of tyrosine. Tyrosine phenol-lyase catalyses the reaction of tyrosine to phenol, pyruvic acid, and ammonia, see Scheme 9. The tyrosine was labelled in the β-position via a multienzymatic synthetic route and the source of the enzyme tyrosinase was the bacterium *Citrobacter freundii*. The mechanism of this enzymatic reaction has been studied by several groups.[52] First the tyrosine is bound to pyridoxal 5-phosphate in the active site. The α-proton of the tyrosine is then abstracted by a basic group on the enzyme with the formation of a quinoid structure. The next step is an enzyme base promoted tautomerization of the phenol ring to a cyclohexadienone and then the activated carbon–carbon bond breaks, see Scheme 10. It has earlier been concluded that the α-proton abstraction is a partially rate-limiting step. A significant $^{11}C/^{14}C$ KIE of 1.067(9) was determined,[53] suggesting that the carbon–carbon bond breaking is at least partially rate-limiting.

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