Fluorine Kinetic Isotope Effects

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Kinetic isotope effects (KIEs) are important as "mechanistic tools" yielding information on rate-limiting steps in complex kinetics and on the structure of transition states (TS) for elementary reaction steps.[1] Hydrogen and carbon are by far the most commonly employed elements in KIE studies, but oxygen, nitrogen, chlorine, and a few others have also been used to some extent.

We would now like to report the first experimentally determined fluorine kinetic isotope effect. The $^{18}F/^{19}F$ KIE for the aromatic nucleophilic substitution (S_NAr) reaction of 2,4-dinitrofluorobenzene with piperidine in tetrahydrofuran (THF) at 22 °C (see Scheme I) has been determined to be 1.0262 ± 0.0007 (n = 3, n = number of experiments for calculation of the mean value).

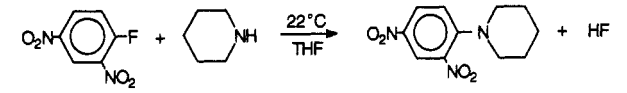
Isotope effects for the element fluorine have not been reported previously despite their great potential value in mechanistic organic and bioorganic chemistry. This is obviously due to the fact that natural fluorine consists entirely of the isotope ^{19}F. However, natural fluorine may be used in combination with the accelerator-produced short-lived radionuclide ^{18}F ($t_{1/2}$ = 110 min) to determine KIEs. We have earlier introduced[2a] the short-lived radionuclide ^{11}C ($t_{1/2}$ = 20.4 min) in combination with ^{14}C in the study of carbon KIEs for a variety of reactions including aliphatic nucleophilic substitution,[2] proton transfer,[3] and an enzymatic reaction.[4]

The S_NAr reactions of the type of substrate used in the present study are of current mechanistic interest[5] and are believed[6] to proceed with rate-limiting carbon–fluorine bond-breaking in aprotic solvents. 2,4-Dinitrofluorobenzene (DNFB) was therefore considered to be a good candidate in the attempt to determine a fluorine KIE, since the nitro groups are activating and the reaction proceeds rapidly, in contrast to the usually slow substitution of fluorine bonded to carbon. Among the aprotic solvents earlier used,[6] an appreciable KIE is most likely to be observed in a non-hydrogen-bonding one such as THF, which was chosen in this study.

The method developed is based on HPLC fractionation of a series of reaction samples with predetermined extents of reaction. Quantitative data are obtained by integration of the UV-detector signals and liquid scintillation counting of the collected radioactive reactant (DNFB) fractions.

The synthesis of the radiolabeled substrate[7] was accomplished by an exchange reaction of DNFB with (^{18}F)fluoride.[8] In the KIE experiments, five capped vials each containing 1 mL of

Scheme I



solutions of different concentrations of piperidine in THF were prepared. These were then thermostated, and 0.2 mL of the DNFB solution was added to each vial. DNFB was in excess, yielding extents of reaction up to 70%. After complete reaction the samples were diluted so that a linear UV-detector response would be obtained, and the samples were analyzed by HPLC.[9] The reactant fraction was collected in scintillation bottles containing 15 mL of scintillation cocktail. The radioactive fluoride formed in the reaction could be removed by a solid-phase extraction[10] before the HPLC injection, or the mixture could be applied directly to the HPLC column. Since no significant difference could be detected in the results from these two methods, the simpler direct HPLC procedure was followed. Careful analysis of the eluent did not reveal any significant amount of radioactivity passing through the column. The radiochromatograms showed only the reactant peak (retention time, t_R 2.45 min). In the UV chromatogram, signals for toluene (t_R 4.48 min) and the product N-(2,4-dinitrophenyl)piperidine (t_R 5.78 min) were observed in addition to the reactant peak. The ^{18}F radioactivity of the samples was measured by liquid scintillation counting.[11] The ^{18}F CPM (counts per minute) values were corrected for background radioactivity, radioactive decay, and dilution (by weight). The fraction of reaction for radioactive DNFB, f_{18}, was calculated as the ratio $C(x)/C(0)$ for each sample, where $C(x)$ is the corrected CPM value for unreacted [^{18}F]DNFB at x% reaction and $C(0)$ is the corrected CPM value for [^{18}F]-DNFB at 0% reaction (no piperidine added). The fraction of reaction for (^{19}F)DNFB, regarded as equivalent to the total fraction of reaction,[12] was determined by dividing the ratio of reactant and internal standard UV areas for each sample with that obtained for 0% reaction. Each sample was analyzed five times, and the mean value from these determinations was used in the calculations. The KIE was calculated as the mean value of the KIE at each point according to the following equation, which is valid when the reaction is of first order in the labeled reactant:[13]

$$k_{18}/k_{19} = \ln(1-f_{18})/\ln(1-f_{19})$$

The results obtained from three such kinetic experiments were 1.0274 ± 0.0053 (n = 5), 1.0260 ± 0.0064 (n = 4), and 1.0251

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(4) Axelsson, B. S.; Bjurling, P.; Matsson, O.; Långström, B. *J. Am. Chem. Soc.* **1992**, *114*, 1502.
(5) See, *e.g.*: (a) Terrier, F. *Nucleophilic Aromatic Displacement. The Influence of the Nitro Group*. VCH: New York, 1991. (b) Forlani, L.; Bosi, M. J. *J. Phys. Org. Chem.* **1992**, *5*, 429. (c) Akinyele, E. T.; Onyido, I.; Hirst, J. *J. Phys. Org. Chem.* **1990**, *3*, 41. (d) Nudelman, N. S. *J. Phys. Org. Chem.* **1989**, *2*, 1.
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(7) A study of ^{18}F incorporation in DNFB has been reported earlier: Korguth, M. L.; DeGrado, T. R.; Holden, J. E.; Gatley, S. J. *J. Lab. Comp. Radiopharm.* **1988**, *25*, 369.
(8) The ^{18}F was obtained as (^{18}F)fluoride (specific activity 185 GBq/mmol) in ^{18}O-enriched water at the PET center at Uppsala University. The solvent was changed to acetonitrile by azeotropic distillation. A 1-mL portion of a solution of DNFB in acetonitrile (0.1 M) was mixed with 1 mL of a solution containing (^{18}F)fluoride, kryptofix (6.2 mg, 0.0165 mmol), and potassium carbonate (2.3 mg, 0.0166 mmol) in acetonitrile. After 2 min of reaction, the solution was put on silica and the product was eluted with acetonitrile. After evaporation of the solvent under reduced pressure, 1 mL of THF containing toluene as an internal standard was added to yield a final DNFB concentration of 0.02 M. The concentration was determined by HPLC (UV detection) using a calibration curve.
(9) An HP 1084 chromatograph with a UV detector and a radiodetector was used. The chromatograph was equipped with a fraction collector. The column was packed with Nucleosil RP C-18, 5 μm. The mobile phase was 0.01 M ammonium formate, pH 3.5, and methanol 40:60 (v:v), isocratic flow 2.00 mL min^{-1}. The wavelength used was 254 nm, using 430 nm as reference. A UV calibration curve was obtained by plotting integrated detector response against different concentrations of DNFB solutions injected.
(10) Waters SEP-PAK Silica.
(11) The radioactivity counting was performed using a liquid scintillation counter, LKB 1214.
(12) The relative amount of labeled DNFB is less than 0.01% in the reaction solution.
(13) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley and Sons: New York, 1980; Chapter 4.2.1.

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± 0.0074 ($n = 2$). The mean value of these experiments is 1.0262 ± 0.0007. This value is of the same order of magnitude as might be expected from an estimate[14] of the maximal $^{18}F/^{19}F$ KIE for complete loss of zero-point energy for a simple two-center model of a C–*F bond, which yields a value of 1.032 for a typical aromatic C–^{19}F stretching frequency of 1250 cm^{-1}. The magnitude of the observed fluorine KIE is thus suggestive of substantial C–F bond-breaking in the rate-limiting step of the reaction and gives support to the earlier mechanistic conclusion by Nudelman[6] based on a study of the dependence of reaction rate on amine concentration in different solvents.

The use of short-lived radionuclides in reaction mechanism studies has become possible mainly because of the development of drugs and other substances, labeled with short-lived positron emitters such as ^{11}C and ^{18}F, which are used in biomedical research and clinical practice, especially in connection with positron emission tomography (PET).[15] Quite a large number of labeled compounds of this type may be prepared today thanks to the recent development of rapid labeling synthesis.[16]

We conclude that primary fluorine isotope effects may be measured with mechanistically significant precision with our approach using the short-lived radionuclide ^{18}F. Further studies of fluorine KIEs for this and other reaction systems, including enzymatic, are in progress in our laboratory.

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(15) See, *e.g.*: *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain and Heart*; Phelps, M., Mazziotta, J., Schelbert, H., Eds.; Raven Press: New York, 1986.

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