

Lastly, the observed increase in  $[O_2]$  during the "steady-state" phase in Figure 5 is believed to be accompanied by a decrease in the NADH concentration. In the context of model A, this change in  $[NADH]$  implies changing the values of the pseudo rate constants used for certain reactions where NADH is involved. This could destabilize a steady state and lead to oscillations after

$[NADH]$  has decreased below a critical level.

**Acknowledgment.** This research was supported by the Danish Natural Science Research Council.

**Registry No.** NADH, 58-68-4;  $O_2$ , 7782-44-7;  $H_2O_2$ , 7722-84-1; peroxidase, 9003-99-0.

## A Secondary $^{11}C/^{14}C$ Kinetic Isotope Effect in the Base-Catalyzed Prototropic Rearrangement of 1- to 3-Methylindene

B. Svante Axelsson, Kjell-Åke Engdahl, Bengt Långström, and Olle Matsson\*

Contribution from the Department of Organic Chemistry, Institute of Chemistry, Uppsala University, P.O. Box 531, 721 21 Uppsala, Sweden. Received December 19, 1989

**Abstract:** The secondary  $^{11}C/^{14}C$  kinetic isotope effect (KIE) has been determined to  $1.010 \pm 0.005$  for the base-catalyzed rearrangement of 1-methylindene (1) to 3-methylindene (2) isotopically substituted in the methyl group in the solvent benzene at 20 °C by using the tertiary amine DABCO as catalyst. The result is discussed in connection with previously reported values of the corresponding primary  $^2H$  and secondary  $\beta\text{-}^2H_3$  KIEs (5.03 and 1.103, respectively). The isotope effects are consistent with a late TS, structurally close to the ion-pair intermediate postulated in the rate-determining proton abstraction step of the reaction. The influence of hyperconjugation and rehybridization on the magnitude of the secondary carbon KIE is discussed.

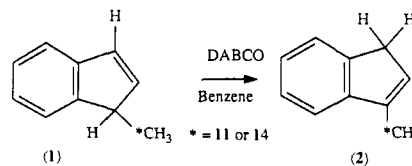
### Introduction

Kinetic isotope effects (KIEs) provide one of the most powerful tools in the elucidation of reaction mechanisms and transition-state structures.<sup>1</sup> KIEs obtained by isotopic substitution at several positions in a reacting system (successive labeling) are especially useful, particularly in combination with theoretical model calculations.

Heavy element isotope effects are relatively small in magnitude, e.g., primary  $^{12}C/^{14}C$  isotope effects are usually less than 16%.<sup>1</sup> Recently the  $^{11}C/^{14}C$  kinetic isotope effect method was presented.<sup>2</sup> This was the first reported application of  $^{11}C$  in isotope effect studies. In the determination of  $^{11}C/^{14}C$  KIEs a large mass range of carbon isotopes is utilized, and the  $^{11}C/^{14}C$  KIE method might thus be suitable for measurements of small effects such as secondary isotope effects.  $^{11}C$  is an accelerator-produced positron emitting radionuclide with a half-life of 20.34 min. The  $^{11}C/^{14}C$  KIE method<sup>3</sup> is based on HPLC fractionation of the labeled reactants and products of the reaction and subsequent radioactivity measurements of  $^{11}C$  and  $^{14}C$  in these fractions as determined by liquid scintillation counting.

Indene and alkylindenes were found to be useful substrates early on for the study of base-catalyzed proton-transfer reactions; a new feature being the first discovery of intramolecular and stereospecific isomerization (with suprafacial shift of the proton), under suitable circumstances, when amines were used as catalysts.<sup>4</sup> As expected, KIEs<sup>5</sup> and enantioselectivity<sup>6</sup> were observed. We have recently reported comprehensive KIE studies by using modern techniques<sup>7,9</sup> and, to the best of our knowledge, the first example

### Scheme I



of an enantiomer-dependent KIE in an asymmetric catalytic reaction.<sup>8</sup>

For the base-catalyzed prototropic rearrangement of 1-methylindene to 3-methylindene the primary deuterium KIE was determined to  $5.03 \pm 0.06$  in toluene at 20 °C by using 1,4-diazabicyclo[2.2.2]octane (DABCO) as the base<sup>7a</sup> (deuterium was substituted for hydrogen in the 1- and 3-positions). The secondary  $\beta$ -deuterium KIE for the same reaction (deuterium substitution in the methyl group;  $-\text{CD}_3$ ) was determined to  $1.103 \pm 0.001$ , in toluene at 20 °C.<sup>7a</sup>

In this paper the secondary  $^{11}C/^{14}C$  KIE for the prototropic rearrangement of 1- to 3-methylindene, catalyzed by DABCO in benzene at 20 °C, see Scheme I, is presented.

Three KIEs, with the isotopic substitution in different positions, have thus been determined for the same reaction. Taken together, these isotope effects yield information on the structure of the TS with regard to its position along the reaction coordinate. Moreover, the methyl KIEs may be used to probe the degree of electronic charge localization on the carbon atom from which the proton is abstracted.

Theoretical model calculations of KIEs for the present reaction, with use of the semiclassical BEBOVIB IV program,<sup>10</sup> are in progress and will be reported in a forthcoming paper.<sup>11</sup> Some of the results

(1) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; John Wiley and Sons: New York, 1980.

(2) Axelsson, B. S.; Långström, B.; Matsson, O. *J. Am. Chem. Soc.* **1987**, *107*, 7233.

(3) Axelsson, B. S.; Matsson, O.; Långström, B. *J. Phys. Org. Chem.* In press.

(4) Bergson, G.; Weidler, A.-M. *Acta Chem. Scand.* **1963**, *17*, 1798.

(5) Bergson, G.; Weidler, A.-M. *Ibid.* **1964**, *18*, 1498.

(6) Ohlsson, L.; Wallmark, I.; Bergson, G. *Ibid.* **1966**, *20*, 750.

(7) (a) Matsson, O. *J. Chem. Soc., Perkin Trans. II* **1985**, 221. (b) Hussénius, A.; Matsson, O.; Bergson, G. *Ibid.* **1989**, 851.

(8) Matsson, O.; Meurling, L.; Obenius, U.; Bergson, G. *J. Chem. Soc., Chem. Commun.* **1984**, 43.

(9) Bergson, G.; Matsson, O.; Sjöberg, S. *Chem. Scr.* **1977**, *11*, 25.

(10) Sims, L. B.; Burton, G.; Lewis, D. E. BEBOVIB IV, *Quantum Chemistry Program Exchange*, 1977; Program No. 337, Department of Chemistry, Indiana University, Bloomington, IN 47401.

from these calculations are briefly summarized and utilized in the discussion below.

### Experimental Section

**General Methods.**  $^{11}\text{C}$  was obtained as  $^{11}\text{C}$  carbon dioxide at the Tandem van der Graaff accelerator at The Svedberg Laboratory, University of Uppsala, by the  $^{14}\text{N}(p,\alpha)^{11}\text{C}$  nuclear reaction on a nitrogen gas target, and trapped in 4-Å molecular sieves placed in a lead-shielded glass oven.

HPLC analyses were performed by using a Hewlett Packard 1084 HPLC liquid chromatograph, equipped with a  $\beta^+$ -flow detector<sup>12</sup> in series with a diode array UV detector. The HPLC was equipped with a fraction collector, Hewlett Packard 79825A, slightly modified by removing the Teflon insert. The columns used were (A) 200 × 4.6 mm, Mino RPC, C18/C2, 5  $\mu\text{m}$  (Pharmacia) and (B) 350 × 4.6 mm, packed at the institute with RP-C18, ODS1, 10  $\mu\text{m}$  (Supelco). The mobile phase was ammonium formate buffer, pH 3.5/methanol, for column (A) 45/55 (v/v) and for column (B) 40/60 (v/v). The injection volumes were 3–20  $\mu\text{L}$ . The HPLC fractions were collected in 20-mL glass scintillation bottles containing 16 mL of scintillation liquid (Zinsser, Quick Scint 1), and the volume of the HPLC fractions was 4 mL. The semipreparative HPLC purification was performed by using a Waters M-45 pump and Dual Path Monitor, UV-2 (Pharmacia Fine Chemicals) UV detector, wavelength 254 nm: column, 250 × 10 mm, RP-C18, 30  $\mu\text{m}$  (Nucleosil), the flow rate was 6 mL  $\text{min}^{-1}$ . The mobile phase was 0.5 M ammonium formate pH 3.5/methanol, 40/60 (v/v).

In the preparative gas chromatography (GC) purifications a Varian Aerograph, Model 90-P, gas chromatograph was used with helium as carrier gas, gas flow: 90–100 mL  $\text{min}^{-1}$ . The detectors used were the TC detector of the chromatograph and a simple GM (Geiger-Müller) instrument as the radiodetector.

The GC analyses were performed by capillary GC by using a GC Varian 3400 gas chromatograph: column, 30 m × 0.32 mm, liquid phase DB-5, film thickness 0.25  $\mu\text{m}$ ; temperature gradient, 50–300 °C, 10 °C  $\text{min}^{-1}$ .

The reaction temperature was kept constant with a proportional regulating thermostat (HETO), with a regulating accuracy of  $\pm 0.005$  °C. The temperature of the water in the thermostat during any kinetic experiment did not deviate more than  $\pm 0.01$  °C, as measured with a calibrated thermometer with an accuracy of  $\pm 0.01$  °C.

The radioactivity counting was performed by using a liquid scintillation counter, LKB 1214. The energy window for the  $^{11}\text{C}$  ( $E_{\text{max}} = 0.98$  MeV) measurements was set to 1–2000 keV (channels: 5–1024) and for the  $^{14}\text{C}$  ( $E_{\text{max}} = 156$  keV) measurements to 1–160 keV (channels: 5–650). The counting time was 1 min for the  $^{11}\text{C}$  and 5–60 min for the  $^{14}\text{C}$  measurements.

A  $^{14}\text{C}$  quench calibration curve was produced. The calibration points were determined by adding small volumes (0.25–1 mL) of the mobile phase of the HPLC system to a scintillation bottle containing a  $^{14}\text{C}$  standard capsule (internal standard kit for liquid scintillation counting, LKB, 117000 DPM) and 16 mL of scintillation liquid. The total volume of added mobile phase ranged from 1 to 4.5 mL. In between two mobile phase additions the radioactivity of the sample was measured, and a quench parameter value (by the external standard method) was obtained. The curve was drawn by plotting the obtained quench parameter values against the counting efficiency for each quench point. The volumes added were chosen so that the values of the quench parameter were close to the quench parameter values of the samples from the kinetic experiments.

All glassware used in the kinetic experiments was cleaned in chromic acid and then rinsed with water, 2 M sodium hydroxide, 2 M hydrochloric acid, and distilled water, followed by drying at 130 °C overnight. The syringes were cleaned in the same way as for the glassware and were finally rinsed with ethanol and ether. The syringes were dried by heating to 55 °C in a vacuum desiccator at 0.05 mmHg for more than 2 h. The glassware and syringes were stored in vacuum desiccators over bluegel under nitrogen atmosphere. Before use, the syringes were repeatedly rinsed with the solvent to be used.

**Materials.** The following were used: methyl iodide, zur Synthese, Merck, purity 99.5% (determined by capillary GC) and tetrabutylammonium hydrogen sulfate (QHSO<sub>4</sub>), Labkemi AB (Sweden). Benzene, pro analysi (p.a.), Merck, purity 99.7%, was dried over 4-Å, freshly prepared, molecular sieves. Methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>), for spectrometry, Merck, was dried over 4-Å molecular sieves. Hydriodic acid (HI), concentrated (57%), was freshly distilled at atmospheric pressure, bp 126.5–127.5 °C. Tetrahydrofuran (THF), Merck, p.a., was freshly

distilled from sodium/benzophenone under nitrogen gas atmosphere. Lithium aluminum hydride (LAH), zur Synthese, Merck, 1-g tablets, purity >98% was used. A 1 M solution of LAH in THF was prepared by cutting a LAH tablet under nitrogen gas atmosphere, ca. 0.2 g of LAH was added to 10 mL of THF in a vial, equipped with a septum screw cap, and kept under nitrogen gas atmosphere overnight. Toluene, p.a., was distilled under nitrogen gas (bp 111 °C) and stored over 4-Å molecular sieves. *n*-Hexane, for spectrometry, Merck, was dried over 4-Å molecular sieves. 1,4-Diazabicyclo[2.2.2]octane (DABCO), purum, Fluka AG,  $\geq 97\%$ , was dissolved in toluene. The solution was distilled under nitrogen gas, in a flask equipped with a Dean-Stark water separator, until no more water separated. The solution was then concentrated and left at room temperature overnight. The crystals formed were filtered off and recrystallized twice from *n*-hexane. The product (mp 159–161 °C) was kept under reduced pressure at 40 °C for 2 h, and was stored under nitrogen gas. A 0.543 M solution of DABCO in toluene was prepared in a 5-mL septum-capped volumetric flask.

**Indene.** Indene, purum, Kistner (Sweden), purity ca. 98%, was fractionally distilled at 9 mmHg, and the fraction boiling between 60 and 61 °C was collected. The distilled indene was further purified by preparative gas chromatography, column: 2.0 m × 3/8 in. packed with 20% Carbowax 20 M, on Chromosorb W, 80/100 mesh. The temperatures of injector and detector were 190 °C and for the column 160 °C. The indene was injected neat, in sample volumes of 100–200  $\mu\text{L}$ , and the fractions were collected in ice-cooled glass traps and stored under helium gas in the freezer. The purified indene was analyzed by capillary GC.

**[ $^{14}\text{C}$ ]Methyl iodide.** [ $^{14}\text{C}$ ]Methyl iodide, 1.85 MBq, 2.0 GBq  $\text{mmol}^{-1}$ , Amersham, at delivery the purity was 98%. The [ $^{14}\text{C}$ ]methyl iodide was delivered in a break-seal ampoule. The opened glass joint part was carefully washed with 2 M sodium hydroxide, 2 M hydrochloric acid, distilled water, and absolute ethanol and was then dried with a hot-air blower. A clean, dry, Teflon covered magnetic stir bar was carefully positioned on the break-seal hook, and the ampoule was connected to a glass vacuum line. The line was connected to the vacuum pump, and vacuum (0.1 mmHg) was applied for about 30 min while the glass line was heated with a hot-air blower. The vacuum line was filled with dry nitrogen gas, and then vacuum was again applied to the line. This procedure was repeated three times. To a 10-mL flask, connected to the vacuum line, 1 mL of dry methylene chloride was added, under nitrogen gas, and the flask was cooled to –100 °C in an ether/N<sub>2</sub> bath. Finally, the vacuum line was evacuated, and the valve to the pump was then closed. The magnetic stir bar was used as a hammer on the break-seal hook by lifting and releasing the bar by means of an external magnet. The ampoule was heated with a hot-air blower to facilitate the distillation of the [ $^{14}\text{C}$ ]methyl iodide to the cooled methylene chloride. After about 3 min the line was filled with nitrogen gas and the flask containing the solution was capped with a septum and kept under nitrogen gas, over bluegel in the freezer.

**1-[ $^{14}\text{C}$ ]Methylindene.** In a 4-mL vial, equipped with a septum screw-cap, 88 mg (0.26 mmol) of QHSO<sub>4</sub> was dissolved in 0.30 mL of 2 M (0.6 mmol) sodium hydroxide and heated to 35 °C. Indene (55  $\mu\text{L}$  = 0.47 mmol) was added to the solution which was stirred magnetically. After about 1 min the solution attained a dark purple color. At this point the methylene chloride solution containing [ $^{14}\text{C}$ ]methyl iodide (with 2  $\mu\text{L}$ , 30  $\mu\text{mol}$  methyl iodide added), heated to 35 °C, was added. The reaction mixture was stirred vigorously. After 4.5 min the reaction was stopped by injection of 1.0 mL of 2 M hydrochloric acid and cooled to +4 °C, and the vial was cooled in an ice bath. After a few minutes at room temperature the methylene chloride phase was removed via syringe and subjected to semipreparative HPLC. Two injections were made, injecting half of the methylene chloride phase, mixed with 0.5 mL of the mobile phase, each time. The fractions containing 1-[ $^{14}\text{C}$ ]methylindene were collected in glass bottles and combined to give a total volume of 40 mL. This solution was extracted with 2 × 1 mL of hexane, and the hexane phases were then combined and concentrated by evaporation to about 1 mL. The 1-[ $^{14}\text{C}$ ]methylindene was then isolated and further purified by preparative GC, column: 2.8 m × 3/8 in., packed with 15% Apiezon on Chromosorb A 40/60 mesh. The temperature of the injector, column, and detector was 90 °C. The hexane phase was injected into the GC, and the 1-[ $^{14}\text{C}$ ]methylindene fraction was collected in an ice-cooled glass trap, containing a small glass wool pad, to prevent formation of an aerosol. The trap was rinsed with 2 × 0.30 mL of benzene, and the solution was transferred to a 1.5-mL septum-capped vial which was kept over bluegel under nitrogen gas in the freezer. This benzene solution was fractionated by HPLC, and the  $^{14}\text{C}$  radioactivity of the fractions was measured by liquid scintillation counting. The specific radioactivity of the 1-[ $^{14}\text{C}$ ]methylindene was approximately 60 MBq  $\text{mmol}^{-1}$ , and the radioactive purity was >99.3%.

**[ $^{11}\text{C}$ ]Methyl iodide.** The [ $^{11}\text{C}$ ]carbon dioxide was released from the molecular sieves on heating the oven and transported by nitrogen gas to

(11) Matsson, O.; Obenius, U. To be published.

(12) Långström, B.; Lundqvist, H. *Radiochem. Radioanal. Lett.* 1980, 42.

a special reaction vessel.<sup>13</sup> It was then trapped in 0.7 mL of 0.5 M LAH in THF. After 1 min reaction time the THF was evaporated, and 2 mL of concentrated hydriodic acid was added. The [<sup>11</sup>C]methyl iodide formed was distilled off, through a drying tower of sodium hydroxide/phosphorous pentoxide 50/50, transported by a nitrogen gas stream to a 1.5-mL vial containing 0.5 mL of methylene chloride, and cooled to -72 °C in a EtOH/CO<sub>2</sub>(s) bath. The radioactivity of this solution was typically 1–2.5 GBq 1 min after the end of the [<sup>11</sup>C]methyl iodide synthesis, the product being of >99% radioactive purity. The specific radioactivity was usually 1–3 GBq μmol<sup>-1</sup>.

**1-[<sup>11</sup>C]Methylindene.** Methyl iodide (2 μL, 30 μmol) was added to the methylene chloride solution containing [<sup>11</sup>C]methyl iodide, and the solution was heated to 35 °C. A solution of 88 mg of QHSO<sub>4</sub> in 0.30 mL of 2 M sodium hydroxide had previously been prepared in a 4-mL vial, equipped with a septum-screw cap. The vial was heated to 35 °C. At the end of the <sup>11</sup>C synthesis, 55 μL (0.47 mmol) of indene was added to the basic solution which was vigorously stirred with a magnetic stirrer. After about 1 min the solution attained a dark purple color, and at this point the methyl iodide solution was added. After 4.5 min the reaction was stopped by injection of 1.0 mL of 2 M hydrochloric acid, cooled to +4 °C. The vial was cooled in an ice bath, and after 1 min at room temperature the methylene chloride phase was removed via syringe and was subjected to preparative GC: column, 1.5 m × 3/8 in., packed with 15% Apiezon L on Chromosorb A, 40/60 mesh; temperatures of injector and detector, 100 °C and column, 95 °C. The 1-[<sup>11</sup>C]methylindene was trapped in an ice-cooled glass trap, prepared with a small glass-wool pad (see above), and the glass trap was rinsed with 0.7–0.8 mL of benzene. The resulting benzene solution was analyzed by HPLC. The specific activity of the 1-[<sup>11</sup>C]methylindene was 300–900 MBq μmol<sup>-1</sup>, and the radioactive purity was always >99%, as analyzed for each synthetic run.

**Kinetic Procedures.** Two different procedures were used, viz. a one-pot procedure and a multiple sample procedure in which the reaction solution was divided between several vials.

**The One-Pot Kinetic Procedure.** The reactant solutions were thermostatted prior to the mixing. In a 2-mL, septum capped, vial 500–800 μL (different experiments) of the 1-[<sup>11</sup>C]methylindene solution and 60–100 μL of the 1-[<sup>14</sup>C]methylindene solution, transferred by syringes, were mixed and thermostatted. The DABCO solution (90–150 μL) was added to the labeled methylindene solution by means of a thermostatted syringe, and the reaction clock was started. The vial was then rapidly shaken before it was placed in the thermostat again, this operation usually taking less than 10 s. The purity of the reactants was analyzed twice by fractionation of a mixture of the <sup>11</sup>C- and <sup>14</sup>C-labeled methylindenes by HPLC. At time intervals the reaction vial was withdrawn from the thermostat, and samples were injected into the HPLC. The reaction time was recorded at the moment of injection. The vial was then quickly thermostatted again, the vial usually being out of the thermostat less than 40 s for each injection. At the injector, the sample-holder was filled with water from the thermostat, just before the injection, to maintain the kinetic temperature of the vial during the injection. The reactants (labeled 1-methylindene) and the products (labeled 3-methylindene) were collected in scintillation bottles. The <sup>11</sup>C radioactivity of these fractions was measured immediately. Since the <sup>11</sup>C radioactivity decreased due to rapid decay, the injection volume was increased to compensate for the decrease in scintillation counts. A kinetic run was continued until the <sup>11</sup>C radioactivity of one fraction had decreased to about 20 000 counts per minute (CPM) chosen as the minimum CPM value for the sake of accuracy (see Results and Discussion).

**The Multiple Sample Procedure.** The start of a kinetic run was performed in the same way as for the one-pot procedure. After the reaction solution had been mixed, the solution was rapidly transferred via a thermostatted syringe to 3–10 vials, about 100 μL to each. The vials were capped and thermostatted. At time intervals vials were withdrawn, and the reaction was stopped by adding 50 μL of concentrated acetic acid. The solution was then fractionated by HPLC, and the radioactivity was measured as described above. In some experiments, several analyses were performed on the same vial after the acid-quenching of the reaction.

## Results and Discussion

The concentrations used in the kinetic experiments were approximately 1–2 mM for 1-methylindene and 85 mM for DABCO. The base-catalyzed prototropic rearrangement of 1- to 3-methylindene follows pseudo-first-order kinetics. The results from eight KIE experiments are summarized in Table I and gave a slope ratio KIE = 1.010 ± 0.005, *n* = 8 (the number of experiments).

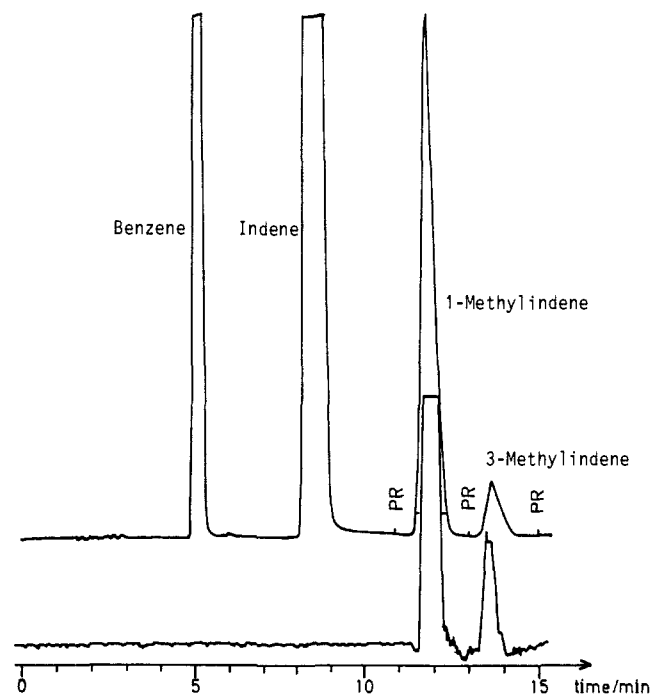
Both of the labeled (<sup>11</sup>C- and <sup>14</sup>C-) 1-methylindenes were synthesized by an extractive alkylation method<sup>14</sup> (according to Scheme II). Both isomeric methylindenes are obtained in the product mixture since 1, which is first formed, is rearranged to

**Table I.** Secondary <sup>11</sup>C/<sup>14</sup>C Kinetic Isotope Effect in the DABCO-Catalyzed Rearrangement of 1- to 3-Methylindene in Benzene at 20 °C

| slope ratio <sup>a</sup><br>KIE | point <sup>b</sup><br>KIE | no. of<br>points | HPLC-<br>system <sup>c</sup> |
|---------------------------------|---------------------------|------------------|------------------------------|
| 1.011                           | 1.014                     | 8                | A                            |
| 1.013                           | 1.013                     | 8                | A                            |
| 1.033                           | 1.016                     | 8                | A                            |
| 1.023                           | 1.012                     | 8                | A                            |
| 0.992                           | 0.996                     | 8                | B                            |
| 1.000                           | 1.002                     | 8                | B                            |
| 1.003                           | 1.002                     | 7                | B                            |
| 1.004                           | 1.016                     | 8                | B                            |

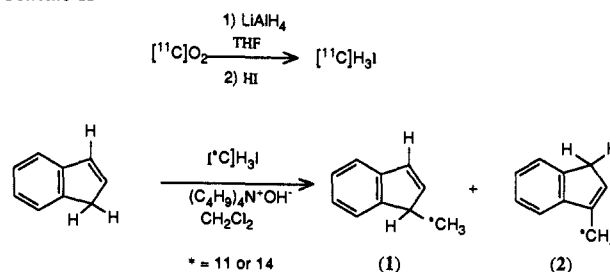
$$x = 1.010 \pm 0.005^d \quad x = 1.009 \pm 0.003^d$$

<sup>a</sup>Calculated as the ratio of the slopes from the plots of  $-\ln(1-f)$  versus reaction time for <sup>11</sup>C and <sup>14</sup>C, respectively. <sup>b</sup>Calculated from eq 3. <sup>c</sup>For an explanation of A and B, see Experimental Section. <sup>d</sup>Standard deviation of the mean.



**Figure 1.** Radio- (lower) and UV-chromatograms for the HPLC separation of 1- and 3-methylindene at the beginning of a KIE study.

## Scheme II

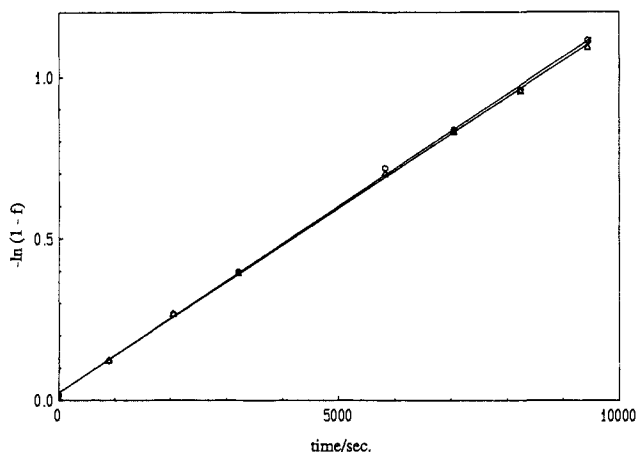


2 under the basic conditions in the synthesis. This creates an optimization problem, with reaction time as an important variable, since the yield of product is affected by the competition between isomerization and radioactive decay on one hand and formation of product on the other. An increase in yield of 15% was achieved by use of a Simplex optimization<sup>15</sup> procedure with reaction time, temperature, and the ratio of tetrabutylammonium hydroxide to substrate as variables. The 1-[<sup>11</sup>C]methylindene was synthesized and purified within 50 min from arrival of the <sup>11</sup>C radioactivity. The radiochemical yield of the synthesis was usually 15–20%, and the radioactivity of the 1-[<sup>11</sup>C]methylindene solution was usually 100–500 MBq, after purification.

**Table II.** Results from One Experiment of the Rearrangement Reaction of 1- to 3-Methylindene, Catalyzed by DABCO in Benzene at 20 °C

| no.,<br>frac <sup>a</sup> | $^{14}\text{C}$ data |                     |               | $^{11}\text{C}$ data |                    |  |             | point KIE <sup>f</sup> |
|---------------------------|----------------------|---------------------|---------------|----------------------|--------------------|--|-------------|------------------------|
|                           | CPM <sup>b</sup>     | SQP(E) <sup>c</sup> | $-\ln(1-f)^d$ | CPM                  | ETIME <sup>e</sup> | CPM <sub>corr</sub> <sup>f</sup> $\times 10^6$ | $-\ln(1-f)$ |                        |
| 1, 1                      | 986.14               | 401.6               | 0.12533       | 187725               | 1878               | 5.4191   | 0.12167     | 1.0044                 |
| 1, 2                      | 7385.7               | 406.1               |               | 186337               | 5553               | 4.1886   |             |                        |
| 2, 1                      | 1963.5               | 403.5               | 0.26896       | 189673               | 3114               | 1.0991   | 0.26730     | 1.0099                 |
| 2, 2                      | 6362.6               | 404.7               |               | 167356               | 5466               | 3.5868   |             |                        |
| 3, 1                      | 2737.2               | 401.4               | 0.39657       | 160196               | 4026               | 1.5479   | 0.39790     | 1.0145                 |
| 3, 2                      | 5623.8               | 404.9               |               | 155234               | 5377               | 3.1674   |             |                        |
| 4, 1                      | 4207.2               | 403.1               | 0.70129       | 123492               | 5286               | 2.3967   | 0.71688     | 1.0289                 |
| 4, 2                      | 4139.5               | 406.8               |               | 123996               | 5195               | 2.2868   |             |                        |
| 5, 1                      | 6536.7               | 403.5               | 0.83110       | 114321               | 6214               | 3.6679   | 0.83597     | 1.0111                 |
| 5, 2                      | 5044.4               | 405.6               |               | 83033                | 6311               | 2.8063   |             |                        |
| 6, 1                      | 9161.7               | 402.6               | 0.95758       | 71920                | 7789               | 5.2263   | 0.95965     | 1.0066                 |
| 6, 2                      | 5706.9               | 403.9               |               | 38608                | 8089               | 3.2445   |             |                        |
| 7, 1                      | 14205                | 403.2               | 1.09398       | 72536                | 8708               | 8.1930   | 1.11388     | 1.0224                 |
| 7, 2                      | 7152.0               | 402.8               |               | 38319                | 8555               | 4.0041   |             |                        |
| O $^{11}\text{C}$ , 1     |                      |                     |               | 799.9                | 98                 | 0.000031                                       | 0.01573     |                        |
| O $^{11}\text{C}$ , 2     |                      |                     |               | 34754                | 196                | 0.019697                                       |             |                        |
| O $^{14}\text{C}$ , 1     | 291.00               | 403.5               | 0.01913       |                      |                    |  |             |                        |
| O $^{14}\text{C}$ , 2     | 15067                | 402.3               |               |                      |                    |  |             |                        |
| $\infty^{14}\text{C}$ , 1 | 9846.8               | 403.8               |               |                      |                    |  |             |                        |
| $\infty^{14}\text{C}$ , 2 | 120.50               | 404.1               |               |                      |                    |  |             |                        |

<sup>a</sup>Reaction point and fraction no. <sup>b</sup>CPM = counts per minute. <sup>c</sup>Scintillation quench parameter. <sup>d</sup>Calculated according to eq 2, see text. <sup>e</sup>Elapsed time from start of radioactivity measurements, used in eq 1, see text. <sup>f</sup>Calculated from eq 1, see text. <sup>g</sup>Calculated from eq 3, see text.



**Figure 2.** A plot of  $-\ln(1-f)$  versus reaction time, for the reaction of  $^{11}\text{C}$ - (O) and  $^{14}\text{C}$ - ( $\Delta$ ) labeled 1-methylindene, respectively, in the DABCO-catalyzed rearrangement reaction.

The time for one HPLC analysis was 12–14 min, and usually 7–8 analyses were performed before the  $^{11}\text{C}$  radioactivity had disintegrated to too low a level for analyses. Representative UV and radiochromatograms are shown in Figure 1.

The KIEs were determined in two series of experiments (four in each), in which two different columns were used in the HPLC separation. The resulting KIEs obtained by using columns A and B (see Experimental Section) were  $1.014 \pm 0.001$ ,  $n = 4$ , and  $1.004 \pm 0.004$ ,  $n = 4$ , respectively. Since no safe conclusions regarding the cause of the deviation could be drawn, the mean value from all eight experiments is used in the further discussion.

The results from one representative kinetic experiment are presented in Table II. The resulting plot, of  $-\ln(1-f)$  versus reaction time, is shown in Figure 2.

Corrections of the  $^{11}\text{C}$  CPM values were performed, by using the equation  $Z = X - (Y + B)$ , where  $B$  is the background CPM value of the  $^{11}\text{C}$  measurements measured next to the fraction,  $Y$  is the  $^{14}\text{C}$  CPM value,  $X$  is the total CPM value, and  $Z$  is the  $^{11}\text{C}$  CPM value. The half-life corrections were then made according to eq 1

$$\text{CPM}_{\text{corr}} = Z / \{0.5 \exp(t/t_{1/2})\} \quad (1)$$

where  $\text{CPM}_{\text{corr}}$  is the decay-corrected  $^{11}\text{C}$  CPM value,  $t$  is the elapsed time (ETIME), in seconds, and  $t_{1/2}$  is the  $^{11}\text{C}$  half-life, 1220 s. The slope KIE was calculated as the ratio of the  $^{11}\text{C}$  and  $^{14}\text{C}$  slopes of the lines  $-\ln(1-f)$  plotted versus reaction time.

The fraction of reaction,  $*f$ , for the  $^{11}\text{C}$  and  $^{14}\text{C}$  reactions, was calculated according to eq 2

$$*f = C_P / (C_R + C_P); \quad * = 11 \text{ or } 14 \quad (2)$$

where  $C_P$  and  $C_R$  are the CPM values for the product and reactant fraction, respectively. The point KIE was calculated according to eq 3

$$^{11}k/^{14}k = ^{11}(Q_i - Q_0) / ^{14}(Q_i - Q_0) \quad (3)$$

where 11 and 14 denotes that the subtraction applies to  $^{11}\text{C}$  and  $^{14}\text{C}$ , respectively.  $Q_i$  is the  $-\ln(1-f)$  value for point  $i$  and  $Q_0$  is the  $-\ln(1-f)$  value for the reactant at  $t = 0$ . This was a correction for radioactive impurities.

There was no detectable difference in results from the multiple-sample and one-pot procedure (see Experimental Section). An experiment was performed in which the reaction in one vial was quenched with acetic acid, and five fractionations were performed and counted for  $^{14}\text{C}$  radioactivity. The mean value of the points did not differ from the line of the one-pot reaction result, in the same experiment. The relative standard deviation of the five points was 0.2%, and for the one-pot experiment the standard deviation of the  $^{14}\text{C}$  slope was 1%, indicating that the accuracy of the determination of the fraction of reaction of one sample in the multiple-sample procedure is better than the accuracy of a full experiment with the one-pot procedure.

Three KIEs, with the isotopic substitution in different positions, have thus been determined for the same reaction. Toluene, in which the deuterium KIEs were determined,<sup>9</sup> was not used as solvent in the  $^{11}\text{C}/^{14}\text{C}$  experiments because of the poor HPLC separations of the toluene solutions compared to those of benzene. It is assumed in the present discussion that the different solvents in these experiments have no or very little effect of the KIE value, e.g., the dielectric constants ( $\epsilon$ ) at 20 °C are 2.38 and 2.28 for toluene and benzene, respectively.

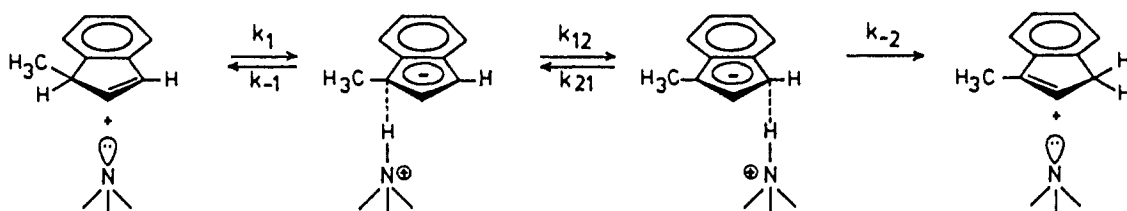
The 1,3-proton transfer in indene and its alkyl-substituted analogues is believed to occur via one or, more likely, two ion-pair intermediate(s).<sup>16</sup> The suggested intermediates are tightly hydrogen-bonded complexes between the protonated amine and the carbanion, with the ammonium ion located above C-1 and C-3 of the indene moiety, respectively.<sup>16</sup> The rearrangement of 1 to

(13) Långström, B. et al. *J. Nucl. Med.* 1987, 28, 1037.

(14) (a) Brändström, A. *Preparative Ion Pair Extraction*; Swedish Academy of Pharmaceutical Sciences, P.O. Box 1136, S-111 81 Stockholm, Sweden. (b) Brändström, A. In *Advances in Physical Organic Chemistry*; Gold, V., Ed.; Academic Press: London, Vol. 15, pp 267–330.

(15) Spendley, W.; Heat, G. R.; Himsworth, F. *Technometrics* 1962, 4, 441.

Scheme III



**2** may be regarded as irreversible.<sup>17</sup> A credible mechanism is shown in Scheme III.

The KIE for the rate-determining ionization step ( $k^H_1/k^D_1$ ) is thus related to the observed KIE ( $k^H/k^D$ ) and the ion-pair collapse ratios ( $\sigma = k_{-1}/k_{-2}$ , L = H or D) according to eq 4.<sup>7b</sup> This equation also contains the ion-pair equilibrium constant  $\chi$ .

$$k^H/k^D = (k^H_1/k^D_1)(1 + \sigma^D\chi^D)/(1 + \sigma^H\chi^H) \quad (4)$$

The collapse ratio of the ion-pair intermediates and  $\chi$  in the rearrangement of **1** are not known. However,  $\sigma$  has been assumed to be much smaller than unity.<sup>17</sup> Under circumstances when this holds and the ion-pair equilibration is fast, the observed KIE can be identified with the KIE on the proton abstraction step. Experiments intended to shed light on the rate of ion-pair equilibration compared to the rate of ion-pair collapse are in progress.<sup>18</sup> The following discussion is based on the assumption that the observed KIE is equal to the KIE on proton abstraction.<sup>7b</sup>

A secondary<sup>19</sup> isotope effect results from an isotopic substitution at a position where no bond formation or breaking occurs. Any isotopically sensitive vibrational frequency that decreases on going from reactant to TS 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). The present <sup>13</sup>C/<sup>14</sup>C isotope effect might have its origin in the hybridization change<sup>19</sup> 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. This rehybridization effect causes a normal secondary KIE since the force constant for this mode is decreased.<sup>19</sup> Other effects that might contribute to the secondary isotope effect are hyperconjugation<sup>19</sup> and inductive effects.<sup>19</sup>

The normal  $\beta$ -deuterium KIE of 10.3% determined for this reaction (DABCO in toluene)<sup>7a</sup> was interpreted as being caused by hyperconjugative and/or inductive interaction between the negative charge on C-1 of the indene ring and the methyl group in the TS.<sup>7a</sup> A simple valence bond or MO picture shows that such anionic hyperconjugation<sup>20a</sup> has the effect of bond lengthening and decreased force constants for the C-L bonds of the methyl group since electronic charge is fed into the lowest vacant group orbital which is antibonding.<sup>20</sup> This interaction will be maximal when the C-C bond is perpendicular to the p-orbital on C-1. Thus two factors related to the position of the TS along the reaction coordinate will tend to reduce the observed KIE as compared to the maximal KIE which should be obtained for the most ion-pair intermediate-like TS: (i) the amount of negative charge on C-1 and (ii) the angle between the C-C bond and the p-orbital carrying the charge. The negative charge available for hyperconjugation will depend on the extent to which charge delocalization in the ring system takes place synchronously with C-H bond breaking. The inductive effect from the negatively charged carbon is also expected to contribute to a normal secondary deuterium isotope effect, perhaps to a smaller extent than the hyperconjugation. This interpretation is consistent with a small variation of the secondary

$\beta$ -deuterium KIE observed when the strength of the catalyzing base is changed.<sup>7a</sup> A corresponding variation was obtained for the primary KIE and the reaction rate.<sup>7</sup> The implications of hyperconjugation with regard to the methyl carbon KIE are less easy to predict. While the strength of the C-L 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.

The results of BEBOVIB<sup>10</sup> model calculations yield inverse or normal secondary carbon KIEs for the present reaction depending on the choice of model for the force field of the methyl group in reactant and TS. The TS force constants were varied with bond order according to a set of empirical equations (Badgers rule, etc.) as is usual in the BEBO approach.<sup>21</sup> The bond orders were obtained from bond distances by Pauling's<sup>22</sup> relation. Inverse isotope effects were obtained when the force constants for stretching and bending vibrations in the methyl group were derived from equilibrium bond distances calculated by the semiempirical MNDO method<sup>23</sup> for 1-methylindene and the corresponding anion as models for reactant and limiting product-like TS, respectively. However, the MNDO method seems to overestimate the C-C bond shortening when going to the anion.<sup>11</sup> Thus the C-C force constants increase in the TS with resulting inverse KIE. This choice of force field also yields secondary  $\beta$ -<sup>2</sup>H<sub>3</sub> KIEs which are small compared to the experimentally determined values, e.g., the KIE calculated for the most product-like TS is ca. 1.06 whereas the experimental values range from 1.082 to 1.103.<sup>7a</sup> In another set of calculations, the same protocol was used except that bond distances C-C and C-H were taken from high level ab initio calculations on ethane<sup>24</sup> and ethyl anion,<sup>25</sup> respectively. Here, the shortening of the C-C bond was less pronounced. Calculations were also performed for a model based on these data, but in which delocalization was simulated by taking a fraction (e.g., 0.3 which is roughly the charge on C-1 in the indenyl anion according to MNDO<sup>11</sup> and other simple quantum chemical estimates<sup>16a</sup>) of the change in bond order between the initial and transition states.

The experimentally determined value of  $k_{11}/k_{14} = 1.01$  corresponds to bond orders of 0.75–0.85 for the forming N-H bond in the model calculations based on the ethane/ethyl anion ab initio data. No agreement was found for the MNDO based model in which the secondary carbon KIE is normal only for very reactant-like transition states. For the deuterium-substituted methyl group, agreement between the experimental value of 1.103 and the theoretically calculated secondary KIE is obtained only for the ab initio based model at bond orders of  $\geq 0.65$  for degrees of delocalization of 0.3 or larger, mimicked as described above. The calculated semiclassical primary deuterium KIE shows agreement with the experimental  $k_H/k_D$  of 5.03 at bond order 0.65, whereas the Bell tunnel corrected KIE matches at 0.7.

### Conclusion

The secondary <sup>13</sup>C/<sup>14</sup>C KIE arising from an isotopically labeled methyl group in the base-catalyzed 1,3-proton transfer in methylindene is normal and fairly high. Within the framework of

(16) (a) Wold, S.; Bergson, G. *Ark. Kemi* **1967**, *28*, 245. (b) Almy, J.; Cram, D. J. *J. Am. Chem. Soc.* **1969**, *91*, 4459.

(17) (a) Meurling, L.; Bergson, G. *Chem. Scr.* **1974**, *6*, 104. (b) Bergson, G.; Ohlsson, L. *Acta Chem. Scand.* **1967**, *21*, 1393.

(18) Hussénius, A.; Matsson, O.; Bergson, G. To be published.

(19) Reference 1, Chapter 6.

(20) (a) von Raguë Schleyer, P.; Kos, A. J. *Tetrahedron* **1983**, *39*, 1141. (b) DeFrees, D. J.; Taagapera, M.; Levi, B. A.; Pollack, S. K.; Summerhays, K. D.; Taft, R. W.; Wolfsberg, M.; Hehre, W. J. *J. Am. Chem. Soc.* **1979**, *101*, 5532 and references therein.

(21) Sims, L. B.; Lewis, D. E. In *Isotopes in Organic Chemistry*; Buncl, E., Lee, C. C., Eds.; Elsevier: 1984; Vol. 6.

(22) Pauling, L. *J. Am. Chem. Soc.* **1947**, *69*, 542.

(23) (a) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899. (b) *Ibid.* **1977**, *99*, 4907.

(24) Pross, A.; DeFrees, D. J.; Levi, B. A.; Pollack, S. K.; Radom, L.; Hehre, W. J. *J. Org. Chem.* **1981**, *46*, 1693.

BEBOVIB model calculations of the isotope effects, the value is consistent with secondary  $\beta$ -deuterium and primary deuterium KIEs determined for the same reaction. The KIEs correspond to a bond order of around 0.7 for the forming N-H bond and a charge fraction of at least 0.3 localized on the carbon atom undergoing bond cleavage in the TS. These results are also consistent with the Brønsted coefficient of 0.79 determined for this reaction by using a series of structurally similar rigid tertiary amine bases including DABCO<sup>26</sup> (in the solvent DMSO), indicating a rather

ion-pair intermediate-like TS.

The methyl group carbon KIE seems to be a promising complement to the secondary  $\beta$ -deuterium KIE in probing anionic hyperconjugation.

**Acknowledgment.** We express our gratitude to P. Malmberg for the radionuclide production. Discussions with Dr. S. Sjöberg and Professor G. Bergson are much appreciated. This work was supported financially by the Swedish Natural Science Research Council.

(25) Spitznagel, G. W.; Clark, T.; Chandrasekhar, J.; von Raguë Schleyer, P. J. *Comput. Chem.* **1982**, *3*, 363.

(26) Meurling, L. *Chem. Scr.* **1975**, *7*, 23.

**Registry No.** <sup>11</sup>C, 14333-33-6; <sup>14</sup>C, 14762-75-5; 1-methylindene, 767-59-9.

## Primary <sup>11</sup>C/<sup>14</sup>C and Secondary <sup>1</sup>H/<sup>2</sup>H Kinetic Isotope Effects in the S<sub>N</sub>2 Reaction of Hydroxide Ion with Methyl Iodide. The Relationship between Different Carbon Isotope Effects

B. Svante Axelsson, Olle Matsson,\* and Bengt Långström\*

*Contribution from the Department of Organic Chemistry, Institute of Chemistry, Uppsala University, P.O. Box 531, S-751 21 Uppsala, Sweden. Received December 19, 1989*

**Abstract:** The short-lived radionuclide <sup>11</sup>C has been used in kinetic isotope effect (KIE) studies. The primary <sup>11</sup>C/<sup>14</sup>C KIE for the reaction of hydroxide ion with labeled methyl iodide in 50% dioxane/water at 25 °C was determined to be 1.192 ± 0.001. A trend of progressively increasing KIE with mass difference is found when this value is compared to previously reported <sup>12</sup>C/<sup>13</sup>C and <sup>12</sup>C/<sup>14</sup>C KIEs. Simple theory predicts an almost linear relationship. The validity of the values of  $r = \ln(k_{12}/k_{14})/\ln(k_{12}/k_{13})$  and  $\ln(k_{11}/k_{14})/\ln(k_{12}/k_{14})$  obtained by a simple theoretical treatment is confirmed by BEBOVIB calculations of the KIEs. Transition-state (TS) models investigated were varied from reactant- to product-like and employed three different types of reaction coordinate movement in the TS: (A) the methyl group moving as one rigid mass unit in the decomposition mode; (B) the methyl hydrogens show Walden inversion the amount of which is independent of TS geometry; and (C) the Walden component is varied with TS geometry and most pronounced for the symmetric TS. The discrepancy between theory and experiment regarding the relation between different carbon KIEs is discussed, but no rationalization is given. The  $\alpha$ -secondary <sup>1</sup>H/<sup>2</sup>H KIE was determined to be 0.881 ± 0.012 and 0.896 ± 0.011 by using <sup>11</sup>C and <sup>14</sup>C as tracers in two double label experiments where  $k_H/k_D$  is calculated from the primary carbon KIE and the observed value of  $k_{11H}/k_{14D}$  or  $k_{11D}/k_{14H}$ , respectively. Comparison of experimental with theoretically calculated primary carbon and secondary deuterium KIEs shows the best agreement for reaction coordinate model C at a bond order between 0.1 and 0.3 for the forming C-O bond. The small discrepancy between the values of the secondary deuterium KIE is discussed in terms of tunneling.

### Introduction

Kinetic isotope effects are powerful tools in the elucidation of organic and enzymatic reaction mechanisms.<sup>1</sup> Especially valuable information can be obtained by isotopic substitution at several positions of the reactants (successive labeling). The transition-state structure can, in favorable cases, be inferred by comparison of experimental results with those from model calculations.

Recently we reported on the first example of a <sup>11</sup>C/<sup>14</sup>C kinetic isotope effect (KIE), determined for the methylation of *N,N*-dimethyl-*p*-toluidine.<sup>2,3</sup> The radionuclide <sup>11</sup>C is an accelerator produced short-lived positron emitter with a half-life of 20.34 min. The <sup>11</sup>C/<sup>14</sup>C method<sup>4</sup> is a one-pot technique based on separation of the labeled reactant and product by HPLC and subsequent radioactivity measurements of the collected fractions by liquid scintillation counting. The combination of <sup>11</sup>C and <sup>14</sup>C in isotope

effect studies might be fruitful since the largest feasible mass range of carbon isotopes is utilized, resulting in relatively large isotope effects.

Introducing the radionuclide <sup>11</sup>C makes four isotopes of carbon available for KIE determinations. At least three different carbon KIEs can thus be measured with reasonable precision. If the experimental values of these carbon KIEs (<sup>11</sup>C/<sup>14</sup>C, <sup>12</sup>C/<sup>14</sup>C, and <sup>12</sup>C/<sup>13</sup>C) were determined for the same reaction, it would be possible to test the theoretical predictions concerning relative strengths of different carbon KIEs.<sup>5,6</sup>

Isotope effects in the reaction of hydroxide ion with methyl iodide have been studied by Bender and Hoeg<sup>7</sup> who determined the <sup>12</sup>C/<sup>14</sup>C KIE to be 1.088 ± 0.010 in 50% dioxane/water at 25 °C, and by Lynn and Yankwich<sup>8</sup> who determined the <sup>12</sup>C/<sup>13</sup>C KIE to be 1.035 ± 0.006 in water at 31 °C. The latter value is smaller than expected from the <sup>12</sup>C/<sup>14</sup>C KIE value using the simple theoretical model presented by Bigeleisen. It has been suggested that this deviation was caused by experimental errors or due to

(1) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; John Wiley and Sons: New York, 1980.

(2) Axelsson, B. S.; Långström, B.; Matsson, O. *J. Am. Chem. Soc.* **1987**, *109*, 7233.

(3) See: Matsson, O. *Abstracts of Uppsala Dissertations from the Faculty of Science*; 1984; p 723 for a preliminary report of the method.

(4) Axelsson, B. S.; Matsson, O.; Långström, B. *J. Phys. Org. Chem.* In press.

(5) Bigeleisen, J. *J. Phys. Chem.* **1952**, *56*, 823.

(6) Stern, M. J.; Vogel, P. C. *J. Chem. Phys.* **1971**, *55*, 2007.

(7) Bender, M. L.; Hoeg, D. F. *J. Am. Chem. Soc.* **1957**, *79*, 5649.

(8) Lynn, K. R.; Yankwich, P. E. *J. Am. Chem. Soc.* **1961**, *83*, 53.