molecules becomes a faster two-dimensional diffusive process of adduct on the surface of growing polymer molecules. Specific experiments to evaluate the size of these changes, and their effect upon the modeling, would be required for any application where it is felt that they may be important.

Conclusions

A simple formalism for including polymerization reactions in detailed reaction modeling has been discovered. It permits accounting for the growth of amount of polymer in the system without increasing the computational burden significantly. Its limitations of size-independent reactivity and linear growth can be tested by global modeling experiments.

Acknowledgment. Our modeling research was supported by NASA–Lewis Research Center, Grant NAG 3-477 and Contract NAS 3-23542, and the Robert A. Welch Foundation.

Registry No. Acetylene, 74-86-2.

Mechanism of Tritium-Atom-Promoted Isotope Exchange in the Benzene Ring: Application to Tritium Labeling of Biologically Important Aryl Compounds

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Reaction of thermal tritium atoms, generated by microwave activation of T_2 gas, with benzene and biphenyl was studied at $\sim$−50 and −196 °C. The saturation reactions (i.e., benzene $\rightarrow$ cyclohexane-t_6) predominated over isotope exchange (i.e., benzene $\rightarrow$ benzene-t) at −196 °C. However, significant exchange labeling occurred at $\sim$−50 °C, with a concomitant reduction in the yields of saturated products. This reversal in labeled product yields at the different temperatures is due, in part, to the faster rate of H expulsion from the intermediate cyclohexadienyl radical at −50 °C and to the increased mobility of the warmer matrix that retards multiple T· reactions with the same aryl molecule by covering up singly tritiated intermediates. The less volatile aryl compound, biphenyl, was labeled in a diffusionally active matrix of either benzene or cyclohexane, whereas it could not be labeled otherwise.

Introduction

The reaction of discharge-activated tritium gas with organic compounds often results in tritium isotope exchange labeling,[1] particularly when the target compound contains the phenyl group.[2] Limited insight as to the labeling mechanism(s) has been provided from earlier ESR and reaction product studies using substituted benzenes. Cyclohexadienyl-type radicals were observed for reaction of activated H_2 gas with polystyrene and substituted polystyrenes,[3,4] L-phenylalanine, L-tyrosine,[5] and benzene itself.[6,7] Both the reaction of atomic hydrogen with several substituted benzenes and the reaction induced by γ-irradiation also resulted in cyclohexadienyl radical formation.[8]

In contrast, the reaction study of atomic tritium with substituted benzenes has been carried out by labeled product analysis.[9] Although the amount of tritium incorporation was often high, most of the label was found in the fully hydrogenated (tritiated) cyclohexyl ring or in the polymeric compounds produced by undesirable side reactions. Little tritium was found in the parent compound. This observation can be easily rationalized when one considers the experimental conditions used. Most T-labeling experiments were carried out by reacting a stream of T· atoms with the upper few monolayers of solid target matrix at −196 °C, such that [T·] >> [target molecules], and therefore, it is not surprising that saturation (a reaction involving several tritium atoms) predominated over substitution (reaction of a single T· per target molecule). For example, reaction of tritium atoms with benzoic acid afforded primarily cyclohexanecarboxylic acid with only small amounts (1–2%) of tritiated benzoic acid.[9] Similarly, reaction of T· with benzene resulted in the formation of significant amounts of cyclohexane-t_6.[10]

In contradistinction to the "saturation labeling" observed in solid matrices, the gas-phase reaction of hydrogen atoms with benzene afforded additional C_6 products such as cyclohexadiene and cyclohexene.[11] This suggests that the saturation-to-exchange ratio of the products may be controllable by use of the appropriate experimental conditions. Little emphasis, however, has been placed on delineating the factors affecting the isotope exchange process with microwave activated tritium gas. Our recent studies reported herein fill this void with evidence for (i) the stepwise addition of T· to the phenyl group and subsequent expulsion of H· and (ii) the superiority of labeling in a "warm", diffusionally active matrix rather than an immobile one at −196 °C.

Experimental Section

Spectral grade benzene and cyclohexane were obtained from Mallinckrodt and were used without further purification. Benzene-d_6, 1,3-cyclohexadiene, and 1,4-cyclohexadiene were purchased from Aldrich Chemical Co. Chromatography standards toluene, ethylbenzene, *n*-propylbenzene, *n*-hexylbenzene, and *o*-, *m*-, and *p*-xylene were obtained from Polyscience Corp. Chromatographic quality cyclohexene was from Matheson Coleman and Bell.

Tritium gas was generated by heating uranium tritide as described earlier.[9] The labeling apparatus used previously[9] was modified (Figure 1) to permit isotope labeling at temperatures where benzene has an appreciable vapor pressure (> −60 °C).

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(1) For example, see: Lee, R.; Ehrenkaufer, R. L.; Wolf, A. P. *J. Labelled Compd. Radiopharm.* **1963**, *13*, 359–62. Hembree, W. C.; Wolf, A. P.; Ehrenkaufer, R. L.; Lieberman, S. *Methods Enzymol.* **1975**, *37*, 313–21.

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(3) Wall, L. A.; Ingalls, R. B. *J. Chem. Phys.* **1964**, *41*, 1112.

(4) Tiedeman, G. T.; Ingalls, R. B. *J. Phys. Chem.* **1967**, *71*, 3092.

(5) Liming, F. G.; Gordy, W. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *60*, 794–801.

(6) Chachaty, C.; Schmidt, M. C. *J. Chim. Phys. Phys.-Chim. Biol.* **1965**, *62*, 527–35.

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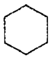
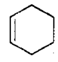
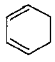
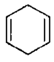
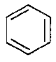
(10) Gordon, B. E.; Otvos, J. W.; Erwin, W. R.; Lemmon, R. M. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 721–24.

(11) Knutti, R.; Bühler, R. E. *Chem. Phys.* **1975**, *7*, 229–43.

0022-3654/84/2088-6266$01.50/0

TABLE I: Reaction Conditions and Products of the Reaction of Benzene with Microwave Activated T_2 Gas

reaction conditions[a]					labeled reaction products, 10^{-4} Ci							
L	temp, °C	*P*	M	*T*	alkanes[b]						*c*	*d*
h_6	−196	0.5	yes	60		1.25				0.034	0.028	
h_6	−196	0.5	no	60	0.086	0.85			0.038	0.068	0.076	
h_6	−196	5.0	no	60		1.30				0.057	0.043	
h_6	−196	5.0	yes	60	0.10	0.77				0.042	0.093	
h_6	−196	5.0	no	60	0.016	0.47	0.049		0.036	1.10[e]	1.98	
h_6	−196	5.0	no	60	0.58	4.90	1.80	0.11	1.96	2.90[e]	0.33	
h_6	−196	5.0	no	6	0.56	1.20				0.096	0.08	
h_6	−53	0.5	no	60		0.60	3.80	0.67	7.0	14.0	1.16	2.0
h_6	−52	0.5	no	60	0.83	1.80	7.60	0.50	10.0	20.0	1.00	2.0
h_6	−49	0.5	yes	60	40.0	21.0	14.5	0.58	10.2	19.0	0.41	1.86
h_6	−60	0.5	yes	15	1.62	1.10	0.54		1.20	4.0	1.41	3.33
d_6	−196	0.5	yes	60		1.29			0.036	0.043	0.032	
d_6	−50	0.5	no	60	77.8	91.0	49.0	3.10	24.0	17.8	0.082	0.74
d_6	−52	0.5	no	60	2.5	4.0	16.8	11.6	24.5	18.7	0.40	0.76
d_6	−51	0.5	yes	60	1.3	4.0	33.0	1.0	15.0	12.0	0.23	0.80

[a] L denotes benzene isotopic substitution; temp, °C is the sample holder temperature; *P* is the T_2 gas pressure in torr (total pressure was always 5 torr with added He moderator); M denotes use of a 1-kG horseshoe magnet placed between the microwave discharge region and the sample holder; *T* is the exposure time (in seconds). [b] "Alkanes" includes all compounds eluted before cyclohexane and may include small alkenes, etc. Yields of C_6 products are also absolute values and given in Curies. Unidentified products with retention times greater than benzene are not given here. [c] Ratio of labeled benzene to the sum of all of the other cyclo-C_6 products including 1,3- and 1,4-cyclohexadiene, cyclohexene, and cyclohexane. [d] Ratio of labeled benzene:1,4-cyclohexadiene. This has been computed only for experiments at −50 °C where an accurate, reproducible ratio can be obtained. [e] In these experiments, the sample matrix was cone-shaped and appeared to "melt" somewhat during the 1-min exposure.

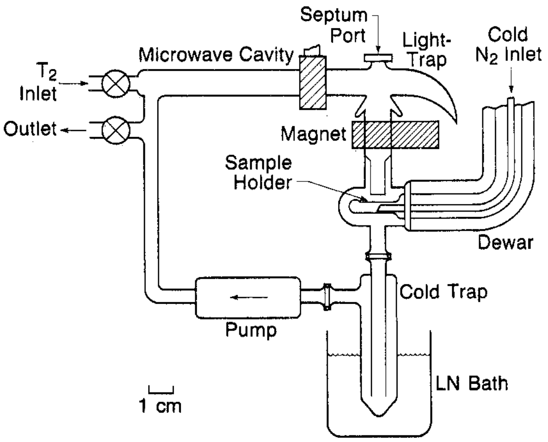


Figure 1. Diagrammatic sketch of the tritiation apparatus. The sample holder temperature was monitored by using a Type K copper constantan thermocouple located inside the sample holder (not shown).

Gaseous benzene was collected in a downstream liquid nitrogen trap so as not to be circulated through the microwave discharge region. The apparatus consisted of a vacuum-tight closed loop with the following features: (i) 12-mm o.d. quartz tubing coupled to an Evenson cavity microwave (2450 MHz) discharge;[9] (ii) a light trap to ensure that the tritiation reactions were not of a photochemical nature; (iii) a >1-kG permanent magnet; (iv) a variable-temperature sample holder capable of holding ~100 μL, (v) a cold trap (−196 °C); (vi) a variable-speed circulatory pump, and (vii) the appropriate inlet and outlet valves for vacuum pumping and gas charging. The labeling apparatus has a volume of ~100 cm^3 such that, when charged with 0.5 torr of T_2 gas, approximately 0.1–0.2 Ci of T_2 was used per experiment. A typical tritium labeling experiment was carried out as follows: The apparatus was evacuated and then repressurized to ~760 torr with helium gas. Exactly 100 μL of spectral grade benzene was injected through the silicon-rubber septum port directly onto the sample holder. The holder was then cooled to −196 °C and the He was evacuated. Approximately 0.5 torr of T_2 gas was admitted, followed by sufficient He to make the final pressure 5 torr. Several experiments were carried out with partial T_2 pressures ranging from 0.05 to 5.0 torr; the total pressure was always 5 torr, with added He if necessary. Pumping was initiated and the sample holder was warmed to ~−50 °C with a stream of precooled (−80 to −50 °C) N_2 gas (Figure 1). After the temperature of the sample holder stabilized (~1–2 min), the glow discharge was initiated with a Tesla coil and maintained at 18 W for 1 min. The discharge and pumping were then ceased, the N_2 coolant gas removed, and the cold finger was charged with liquid nitrogen before pumping the T_2/He mixture into a holding tank. The apparatus was flushed three times with He to remove traces of T_2 and then charged with He to 1 atm. After the sample holder and the trap were warmed to approximately −30 °C, the remaining products in each were washed with cyclohexane into separate vials such that the total volume in each vial was exactly 10 mL. These solutions were then washed 5 times with ~1 M NaCl solution before radiochromatographic analysis was done. Sample activities usually ranged from (0.1–3.5) × 10^{10} dpm or ~(0.5–18) × 10^{-3} Ci per sample. For the more favorable cases, this corresponded to a 10% uptake of the total tritium available.

Gas–liquid radiochromatography (GLRC) as described by Gordon et al.[12] was used to analyze the lighter components of the reaction solutions; most products were separated easily on a 10 ft × $^1/_4$ in. Carbowax column at 60 °C. The polymeric products were not run into the proportional flow counter (PFC) tube and analyzed because sample residues often caused excessively high background counts for subsequent runs. Typically, a 50 mL/min flow of He and a 100 mL/min flow of propane gas was run through the 20-cm^3 PFC tube held at 290 °C. For all experiments a multichannel analyzer (Tracor Northern, TN-7200) in the multiscaling mode was used to record and integrate count rates and peak areas. A dwell time of 2 s/channel was used. Mass and radioactivity peaks were matched by simultaneously recording with a dual-pen strip-chart recorder using [methyl-^{14}C]toluene as a standard. Sample activities were measured by either collecting the desired effluent and subjecting it to liquid scintillation counting (~20% efficiency for "Aquassure" solution) or calibrating the PFC tube with a known activity of tritiated toluene (1939 dpm/μL) and relating the experimentally observed PFC count rates to this standard (calculated efficiency: ~9%).

Results

Reaction of a stream of microwave-activated T_2 gas with a frozen (−196 °C) benzene target resulted in significant sample tritiation. Typically, the target contained (1–2) × 10^{-3} Ci tritium after a 1-min exposure (see Table I). Washing the benzene in 10 mL of cyclohexane with five 25-mL aliquots of ~1 M NaCl

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(12) Gordon, B. E.; Erwin, W. R.; Press, M.; Lemmon, R. M. *Anal. Chem.* **1978**, *50*, 179–82.

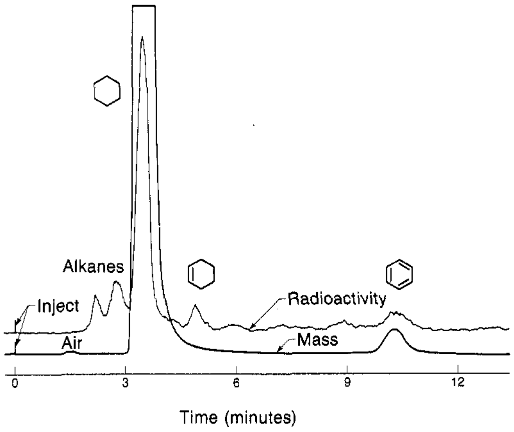

Figure 2. Radiochromatogram of the products obtained from the reaction of tritium atoms with benzene at −196 °C.

solution usually resulted in a 10–50% decrease in sample activity in the organic phase; this was presumably due to the removal of volatile or water-soluble compounds or to the washing-out of traces of TOH formed by reaction of tritium with the hydroxylic surface of the glass apparatus. Generally, increase of the reaction time, microwave discharge power, or T_2 concentration and pressure resulted in greater overall yields of labeled target. None of these variable experimental conditions, however, resulted in significant increases in the yields of labeled benzene. As the reaction time was increased, the overall sample activity approached a limiting value. This type of behavior has been observed earlier, for example, in the reaction of H· with thymine and dihydrothymine.[13] This indicates that the exposed monolayer(s) of the sample became fully reacted (i.e., completely saturated) with tritium. This conclusion is also supported by a comparison of the calculated and experimentally observed sample activities. If we assume that benzene has a molecular diameter of $\sim$5 Å, the 2-cm^2 target surface area has approximately 10^{15} benzene molecules exposed; saturation-labeling of this monolayer results in a calculated tritium uptake of $\sim 3 \times 10^{-4}$ Ci. This is in agreement with the $\sim 1 \times 10^{-3}$ Ci/sample observed herein. Similarly, increasing the microwave discharge power from 9 to 35 W approximately doubled the tritium uptake but did not appreciably change the labeled benzene yield. Under the aforementioned experimental conditions, the major product was tritiated cyclohexane; only small amounts of labeled straight chain hydrocarbons (i.e., hexane or pentane) and labeled benzene were formed. In most experiments, approximately 70–90% of the labeled sample injected onto the GC column could be accounted for by summing the calibrated PFC tube counts and the activity found in the heavier fractions collected (i.e., dimers, trimers, etc.) and assayed for by liquid scintillation counting. Presumably, the remaining activity was lost as immobile polymer retained on the column or due to isotope exchange with the column packing. Of the GC-injected activity, usually less than 10% was found in C_6 products where often >50% of the tritium label was found in the heavier fractions. A typical radiochromatogram is shown in Figure 2.

Effect of Target Temperature. When the sample matrix was warmed to $\sim$−50 °C the benzene vapor ($\sim$0.3 torr) had to be trapped before reaching the microwave discharge region to prevent pyrolysis. This was done by ensuring that the pump (with positive flow from the discharge region to the sample holder) was operating during the warm-up. The effect of warming the sample holder to $\sim$−50 °C was striking. Not only was the overall yield of labeled products increased almost tenfold, but the product ratio of cyclohexane:benzene was reversed (Figure 3). In addition, the partially saturated C_6 hydrocarbons, cyclohexene and 1,3- and 1,4-cyclohexadiene, were formed at −50 °C whereas they were undetected in most experiments at −196 °C. During a 1-min run at $\sim$−50 °C, approximately 5% of the benzene target or $\sim$5 μL was volatilized and collected in the downstream cold trap. This sample fraction was often composed of extremely light compounds containing 1–10 times more activity than that remaining on the sample holder. Although the identity of these radioactive products was not determined, the short GC retention times suggest that the components are light hydrocarbons such as methane, ethane, etc. Experiments are in progress to determine the cracking patterns of aromatic compounds under our experimental conditions. Mixing of the trap and sample holder contents and subsequent GLRC assay demonstrated that the increased labeled benzene yield was not due to the greater fractionation of the lower boiling compounds from the sample holder, but that *more* labeled benzene and *less* cyclohexane was actually formed at $\sim$−50 °C. Tritium incorporation increased rapidly with sample-holder temperature above −60 °C (Figure 4) as did the fraction of tritiated compounds collected in the trap.

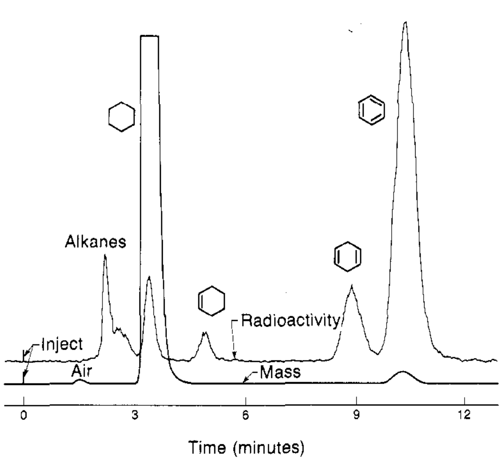

Figure 3. Radiochromatogram of the products obtained from the reaction of tritium atoms with benzene at $\sim$−50 °C.

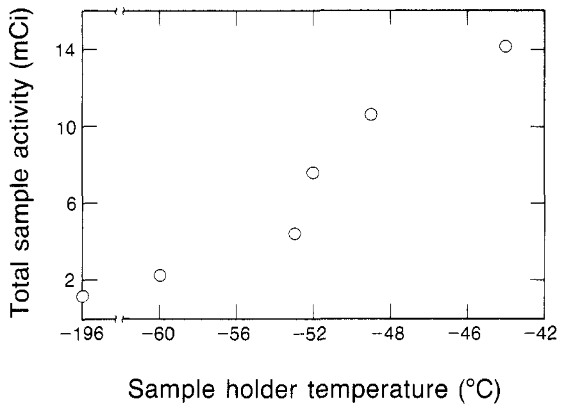

Figure 4. Dependence of sample activity on sample holder temperature. The increase in activity above $\sim$−60 °C suggests that the benzene matrix is relatively immobile below this temperature.

Effect of a >1-kG Magnetic Field. Several experiments at different T_2 partial pressures and sample temperatures were carried out with a 1400-G horseshoe magnet placed between the microwave discharge cavity and the sample holder. Magnetometer measurements showed that the field strength midway between the magnet poles (wherein the stream of discharge-activated T_2 gas was run) was always >700 G. In all experiments the magnet had no observable effect (within the levels of precision observed herein) on either the total sample activity or on the labeled benzene yields. For example, a comparison of experiments carried out at −196 °C and with 0.5 torr of T_2/4.5 torr of He gas mixture showed that the activity and C_6H_5T yield in the absence of the magnetic field (1.50 mCi, 0.45% C_6H_5T yield) was not significantly different from the yield found when the magnet was in place (1.27 mCi, 0.26%). Similarly, experiments run at 5 torr of T_2 pressure with (2.80 mCi, 0.15%) and without (1.06 mCi, 0.54%) the magnet or at higher temperatures ($\sim$−50 °C) with (10.5 mCi, 18.1%) and without (7.7 mCi, 26.0%) the magnet in place gave compa-

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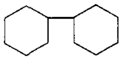
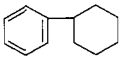
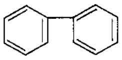
[13] Henriksen, T. *J. Chem. Phys.* **1969**, *50*, 4653–7.

TABLE II: Dependence of Temperature and Matrix for the Reaction of Biphenyl with Microwave Activated T_2 Gas

		absolute product yields, 10^{-4} Ci		
matrix	sample temp, °C			
biphenyl	20	0	0	0
biphenyl	−196	23.9[a]	8.09[a]	0.55[a]
benzene/ biphenyl	−196	3.08	0.243	0
benzene/ biphenyl	−45	2.89	4.55	4.93
cyclohexane/ biphenyl	−48	0.38	0.83	3.70

[a] No correction has been made to account for the use of neat biphenyl in these experiments. These absolute yields, therefore, should be divided by five to get comparative yields with the experiments in which a 20% biphenyl target matrix was used.

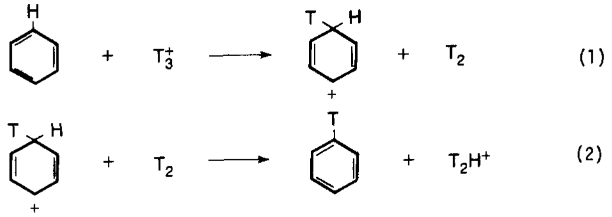
rable overall activities and labeled benzene yields.

Effect of Sample Shape. The geometry of the frozen (−196 °C) target—whether it was made "flat" by injecting 100 μL of benzene onto the sample holder followed by liquid nitrogen addition to the Dewar, or built-up by prior liquid nitrogen addition and subsequent slow (over 5–10 s) dropwise addition of benzene—greatly changed the yields of labeled benzene. Such an observation is difficult to rationalize if the built-up, cone-shaped target remained rigid and unchanged during the course of the reaction; however, it did not. The cone-shaped target appeared to melt slightly and broaden with some loss to its original height. This "melting", or more likely, sublimation, of the cone-shaped target could occur possibly by the warming of the upper target surface due to poor heat conduction. Thus, the benzene surface temperature was probably somewhat warmer than the sample holder temperature. This warmer target was also diffusionally active; i.e., the matrix surface was continuously replenished with previously covered benzene molecules. Comparison of the total activities and labeled-benzene yields for flat and cone-shaped targets showed that the latter had approximately twice the overall sample activity, but ~20 times the labeled benzene yield (Table I). In addition, the partially saturated C_6 derivatives, cyclohexene and 1,3- and 1,4-cyclohexadiene, were found in higher yields for the cone-shaped samples. The definite trend toward greater yields of labeled benzene in these experiments was not masked by the low reproducibility of sample activities caused by the nonuniformity of different cone-shaped targets.

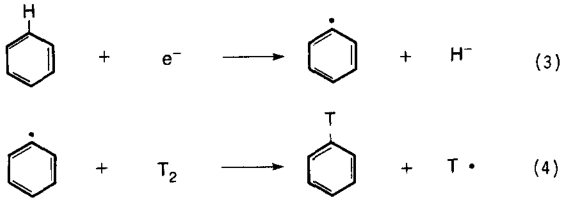
Isotopic Substitution. Experiments carried out with benzene-d_6 in place of benzene-h_6 showed few differences in overall sample activity or tritiated benzene yield. For example, reaction at ~−50 °C of benzene-h_6 with discharge-activated T_2 gas resulted in 18–30% labeled benzene, whereas benzene-d_6 gave 9–26%. These differences in yield are not significant. It is possible, however, that the subtle changes caused by isotopic substitution may be hidden by the large variations in the labeled benzene yields. It was noted that the ratio of labeled benzene:1,4-cyclohexadiene (Y_H) was fairly constant from run-to-run at ~−50 °C ($Y_H = 1.9 \pm 0.06$). Similarly, the corresponding ratio for benzene-d_6 was constant but somewhat smaller ($Y_D = 0.77 \pm 0.02$). Comment on these values is deferred to the Discussion section. A single experiment carried out with a shorter exposure time (15 s) gave a higher value of Y_H ($\simeq 3.3$) indicating that the value of Y_H is probably dependent on the extent of reaction.

Reaction of $T\cdot$ with Biphenyl. Our attempts to label biphenyl at room temperature resulted in severe sample decomposition; no identifiable products were obtained after a 1-min exposure. Earlier attempts to prepare biphenyl radical from biphenyl and hydrogen atoms at room temperature also proved unsuccessful.[8] Yields of labeled biphenyl were also very low when the reaction was carried out at −196 °C (see Table II). In this instance, labeled bicyclohexyl and phenylcyclohexane were identified, but little tritiated biphenyl was found. When $T\cdot$ was reacted at −196 °C with a benzene matrix containing 20% biphenyl, again only the saturated C_{12} products were found. This was not the case, however, when $T\cdot$ was reacted with biphenyl in a benzene or cyclohexane matrix at −50 °C. At this higher temperature, biphenyl was the predominant C_{12}-labeled product (Table II).

Scheme I



Scheme II



Discussion

Reactive Labeling Species. Although it is generally believed that tritium atoms are the sole species responsible for the isotopic labeling of aromatic compounds, it was noted some time ago that many other reactive species, such as ions and excited hydrogen atoms, are expected to exist in the region of a high-energy discharge.[14] Strongly electrophilic cations, such as T_2^+ or T_3^+, or electrons, might then be expected to react with benzene somewhat more readily than neutral tritium atoms (provided they reach the benzene sample), as shown in Schemes I and II. However, that ions are probably not involved in the work reported herein is supported by the numerous literature accounts regarding the reaction of microwave or RF-activated H_2 gas with various organic substrates giving primarily radical products. For example, reaction of hydrogen atoms with 1-butene at −195 °C gave largely the *sec*-butyl radical.[15] Previous ESR studies on the reaction of $H\cdot$ with amino acids and proteins gave a qualitative measure of the chemically stable radicals formed.[16] Bennett and Mile[17] assumed that they had overcome possible ambiguities of radical vs. ion labeling species in their reaction studies of RF-activated H_2 gas with various small molecules by placing a 3000-G magnetic field between the discharge region and the sample target. However, the translational energies of our microwave generated ions are below 1 eV;[18] even at that energy the radius of curvature would be only about 2 cm. Given the short mean free path at 5 torr (ca. 10^{-2} cm) the magnetic field would be of limited effectiveness in removing ions.

Bennett and Mile[17] also identified by ESR measurements several types of free radicals formed in the reaction of $H\cdot$ with organic substrates. Further flow system experiments in which hydrogen atoms were collected at 4 K have demonstrated that $H\cdot$ is the reactive species downstream from the discharge.[19] Hydrogen atoms were also found to exhibit high reactivity with benzene when Anbar et al.[20] showed that the hydrogen atom, unlike the hydrated electron, reacted as an electrophilic agent. Other rate studies since have also demonstrated that $H\cdot$ reacts rapidly with benzene.[11,21]

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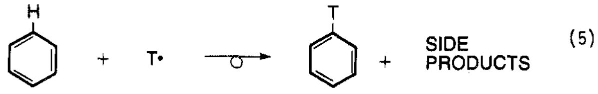
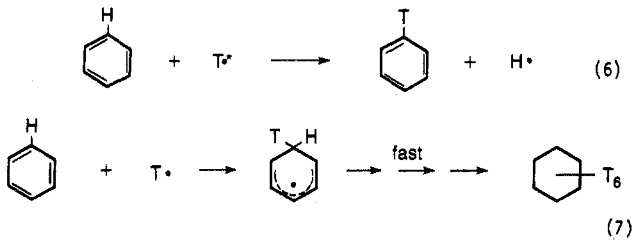
The labeling studies reported here support the general conclusion that hydrogen atoms, not ions, are the reactive species found downstream from the microwave discharge region. Experiments carried out with and without a >1-kg magnetic field between the discharge and sample showed no significant differences in overall activity or benzene yields. Varying the partial T_2 pressure or substituting benzene-d_6 for benzene-h_6 did not change this. These observations then preclude the exchange mechanisms of Schemes I and II. The reaction pathway must then be of the type shown in eq 5.

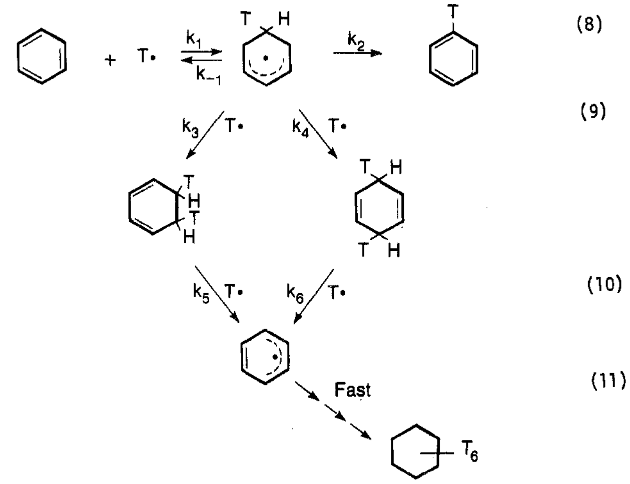


Mechanism of Hydrogen Exchange: Concerted vs. Stepwise. Tritium isotope labeling by T• can occur by either of the following two mechanistic extremes: (i) isotope exchange caused by a hot-atom reaction of tritium (Scheme III, eq 6) with concomitant irreversible stepwise addition of thermal tritium atoms (Scheme III, eq 7) or (ii) tritium exchange as a result of a reversible stepwise addition of tritium (Scheme IV). Scheme III must be incorrect since it has been demonstrated that H• atoms (formed under similar reaction conditions as used here) reach thermal equilibrium over a few centimeters path length.[17,22] Furthermore, the moderator gas He (in which ~1.8 kJ mol^{-1} is removed per collision[23]) also thermalizes T• rapidly under the flow conditions used here. Bennett and Mile[17] have demonstrated that the overall reaction temperature in similar reaction studies (H• + alkenes) may indeed be much less than 25 °C—possibly close to –196 °C. Iodine, which scavenges primarily thermal radicals,[24] was also shown to reduce the overall "crude" activity yields by an approximate factor of ten in reaction of microwave activated T_2 gas and iodobenzene.[25]

Scheme III



Scheme IV



In addition, Scheme III cannot account for the temperature dependence of the product yields reported here. This scheme predicts that the labeled cyclohexane:benzene product ratio should increase with temperature because, although hot atom reaction of T•* is nearly temperature independent, the thermal T• reaction of eq 7 is dependent on temperature (albeit mildly[11,21]). For the experiments reported here, the ratio of cyclohexane:benzene clearly did not increase but decreased dramatically with rising temperature.

Isotope effect measurements also support Scheme IV over Scheme III. A comparison of the labeled benzene:1,4-cyclohexadiene yield ratios (Y_L, L = H or D) gives a rough estimate of the primary isotope effect for labeled benzene formation. The argument for this is as follows. Scheme III should display an overall isotope effect of near unity because the hot atom reaction (eq 6) should have negligible isotope effect and because the addition reactions of eq 7 give only secondary isotope effects for T• addition to benzene and the cyclohexadienyl radical. Evidence for such small isotope effects has been demonstrated in similar reactions such as the H• and D• abstraction from CH_2D_2 by T•, where approximately equal amounts of HT and DT were found,[26] or in the addition reaction of H• with $CD_2{=}CH_2$[17,27] and propylene-d_6.[28] The stepwise mechanism of Scheme IV predicts that the cyclohexane:benzene ratio should decrease at the higher temperatures (k_2 is temperature dependent) and that positive primary isotope effects should be observed. Assuming that the T• addition steps are isotopically insensitive (a secondary isotope effect of unity), such that the yields of H- and D-labeled cyclohexadiene-t_2 are constant, the ratio of k_2^H/k_2^D is given by the ratio $Y_H/Y_D = 2.53 \pm 0.10$ at –50 °C. This isotope effect is too large for a normal secondary isotope effect and must, therefore, be interpreted as a small (but significant) primary isotope effect concordant with Scheme IV. As stated above, this scheme also explains the dramatic effect of changing the sample temperature from –196 to –50 °C. At –196 °C, k_2 is much smaller than either k_3[T•] or k_4[T•] and so the saturation reactions predominate. Elevating the temperature to ~–50 °C has two major effects: (i) increasing the value of k_2 favoring isotope exchange, and (ii) creating a diffusionally active matrix such that the cyclohexadienyl radical formed is rapidly covered up, preventing further reaction with T• (or T_2). This latter point has the effect of reducing the [T•] available for reaction and so k_3[T•] and k_4[T•] are smaller. Active matrix diffusion has been observed in propene matrices at temperatures as low as –196 °C[29,30] and will occur to some extent in a benzene matrix at –50 °C.[31] The faster rates of molecular diffusion in the warmer matrices should, therefore, result in less labeled cyclohexane and more benzene.

Under the experimental conditions used here, little 1,3-cyclohexadiene was formed compared to the yield of 1,4-cyclohexadiene. This is to be expected in that the higher spin densities in the para position of the planar cyclohexadienyl radical[32] should promote T• attack at the 4-position rather than the ortho positions giving $k_4 > k_3$. Secondly, rates at room temperature of H• addition to 1,3-cyclohexadiene are somewhat faster than to the 1,4 isomer,[11] and the difference in rates is expected to be even greater at lower temperatures. This results in $k_5 >> k_6$. The enhanced reactivity of 1,3-cyclohexadiene over the 1,4 compound may be due to the unstable nature of the twisted 1,3 form[33] compared to the planar 1,4 structure.[34] Both of these phenomena ($k_4 > k_3$, $k_5 >> k_6$) contribute to low 1,3-cyclohexadiene yields as compared to 1,4 yields. It is noted that room temperature γ-radiolysis of benzene also afforded more 1,4-cyclohexadiene than the 1,3 isomer.[35]

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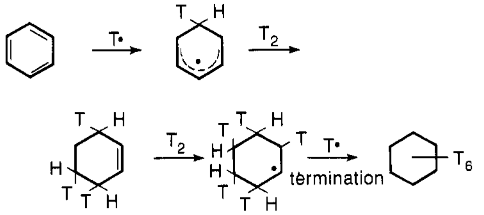
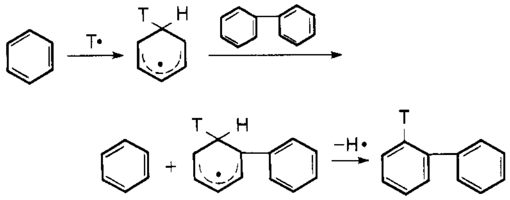
Reaction of either 1,3- or 1,4-cyclohexadiene with additional tritium atoms results in the formation of labeled cyclohexene, another C_6 species found in reasonable quantities at −50 °C (Table I). It is not clear at this time, however, whether T saturation is caused by reaction of multiple tritium atoms or T· initiation followed by radical reaction with T_2 (eq 12). The high T· concentration used, and the greater reactivity of T· over T_2 with C_6 radicals, strongly suggests that the correct mechanism is that of Scheme IV and not that of eq 12.



(12)

Application to Isotope Labeling of Biologically Important Compounds. Isotope exchange labeling can be carried out efficiently if the side reactions leading to saturation (k_3 and k_4 in Scheme IV) are minimized and k_2 (giving substitution) is made faster. It is demonstrated herein that benzene can be labeled in much higher yields by using a diffusionally active matrix. But will this method be general for enhanced labeling of biological molecules that are usually large and nonvolatile? To answer this, a short labeling study for a model of a biological aryl compound—biphenyl—was carried out. Biphenyl may seem, at first, a somewhat unorthodox choice for a "model" in that it does not possess the heteroatom or functional groups typical of biological aryl compounds. It is just this characteristic, however, which makes it very suitable for the purpose of studying the effect of the matrix on labeling of a nonvolatile compound without complicating side reactions. Biphenyl has a low vapor pressure under the experimental conditions used herein, is readily soluble in benzene and cyclohexane, and does not have heteroatom functional groups that may be labeled or displaced by T·. Inspection of Table II shows that biphenyl is successfully labeled at ∼−50 °C in either a benzene or cyclohexane matrix, where other reaction conditions are relatively ineffective. This enhanced labeling is not due to a "dilution effect" as described earlier,[9] in that biphenyl is poorly labeled in a benzene matrix at −196 °C. Enhanced labeling cannot be attributed solely to an increased temperature effect because all attempts to label biphenyl at 20 °C were unsuccessful. It could also be argued that biphenyl may be labeled by the cyclohexadienyl radical in a chain mechanism represented by eq 13.



(13)

This reaction, however, must be of minor significance in that biphenyl is readily labeled in a cyclohexane matrix at −50 °C whereby C_6H_{12} is less expected to undergo reactions of this type. These experiments clearly demonstrate the superiority of labeling in a "warm" matrix where k_2 is greater and the k_3 and k_4 saturation processes of Scheme IV are retarded.

Excitation labeling studies are currently being carried out to determine which matrices offer the highest yield of labeled aromatic compounds with minimal sample degradation. These studies are also being extended to include liquid-phase nonvolatile sample supports that will enhance diffusion of the labeled substrate and, therefore, reduce the yields of saturated side products.

Acknowledgment. This research has been supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant 5 P41 RR01237-02, and by the Department of Energy under Contract DE-AC03-76SF00098. Acknowledgment is also made of the technical assistance of Mr. Raymond G. Aune, and of helpful discussions with Drs. J. W. Otvos and C. T. Peng.

Registry No. T, 15086-10-9; benzene, 71-43-2; biphenyl, 92-52-4.

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