HEAVY-ATOM KINETIC ISOTOPE EFFECTS AND MECHANISM OF THE
ACID-CATALYZED O-SEMIDINE AND P-SEMIDINE
REARRANGEMENTS AND DISPROPORTIONATION
OF 4,4'-DICHLOROHYDRAZOBENZENE

by

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CHAPTER I

INTRODUCTION

Statement of the Problem

Acid-catalyzed benzidine rearrangements have been extensively studied for a long time and much is known about the kinetics and the products of these reactions. Recently Shine and coworkers have measured the heavy-atom kinetic isotope effects (KIE) for benzidine rearrangements and the results have indicated that these intramolecular rearrangements conform with the rules for sigmatropic rearrangements.¹⁻⁶ There are five types of rearrangement in the family of benzidine rearrangements, namely, the benzidine (4,4'), diphenylene (2,4'), o-benzidine (2,2'), o-semidine (N,2') and p-semidine (N,4') rearrangements, which correspond with [5,5]-, [3,5]-, [3,3]-, [1,3]-, and [1,5]- sigmatropic shifts, respectively. KIE studies have been made in most cases among these types of rearrangement, as well as in one case of disproportionation, and only the o-semidine rearrangement has remained unexamined. For example, the two-proton, acid-catalyzed rearrangement of hydrazobenzene (1) into benzidine (2) was found to be a [5,5]-sigmatropic rearrangement, whereas the concomitant rearrangement of 1 into diphenylene (3) was found not to be

a concerted process.^{1,2} Further, the acid-catalyzed rearrangement of 4-methoxyhydrazobenzene (4) into the p-semidine 5 was found to be a concerted [1,5]-sigmatropic shift.³ A concerted [5,5]-sigmatropic process was confirmed in the one-proton, acid-catalyzed rearrangement of 2,2'-dimethoxyhydrazobenzene (6) into 3,3'-dimethoxybenzidine (7).⁴ More recently, it was found that acid-catalyzed and thermal rearrangements of 2,2'-hydrazonaphthalene (8) into 2,2'-diamino-1,1'-binaphthyl (9),⁵ and the acid-catalyzed rearrangement of N-2-naphthyl-N'-phenylhydrazine (10) into the diamine 11⁶ are [3,3]-sigmatropic processes. The hitherto unexamined o-semidine rearrangement (eq. 6) should not be a concerted process, according to rules of orbital symmetry in pericyclic reactions. To confirm that the rules of sigmatropic rearrangement can apply to all types of benzidine rearrangement, the acid-catalyzed reaction of 4,4'-dichlorohydrazobenzene (14)⁷ has now been investigated.

Literature Review

Benzidine Rearrangement and Products

The acid-catalyzed rearrangements of hydrazoaromatics (benzidine rearrangements) have been known for over 100 years⁸ and documented and reviewed⁹⁻¹⁸ in much detail during the last 60 years. The term "benzidine rearrangement" refers to the reaction which aromatic hydrazo compounds undergo, under acid-catalyzed conditions or thermally, to yield compounds having newly formed C-C or C-NH-C bonds connecting the arylamino substituents. The various products formed from hydrazobenzene (1) and related compounds are classified in types, known as benzidine (2), diphenylene (3), ortho-benzidine (22), and ortho- and para-semidine (23 and 24). Also formed are disproportionation products, that is, azobenzenes and arylamines, which accompany rearrangement in most cases.

Intramolecularity

The benzidine rearrangement has been shown to be intramolecular. This was proven by the lack of observed formation of crossed products when two different substrates were rearranged in the same solution. Thus, mass spectrometric measurements showed that in the rearrangement of equimolar amounts of decadeuterio-N-acetylhydrazobenzene (25) and the equivalent undeuterated compound no more than 0.2% of the crossed product could have been produced.¹⁹ Also reaction of ¹⁴C-labeled 2-methylhydrazobenzene (26) in the presence of ortho-hydrazotoluene produced none of the crossed product²⁰.

Other examples of o- and p-semidine rearrangements^{21,22} are shown in equations 11 and 12. In each case crossed products, which might have been formed via intermolecular paths, were not observed.

Thus the benzidine rearrangements are accepted as being intramolecular.

Kinetics

It is recognized and now universally accepted that two types of acid catalyzed benzidine rearrangements exist, commonly called one-proton and two-proton rearrangements. These terms signify that some rearrangements are kinetically first order while others are second order in acid. It is also recognized that a particular rearrangement may at low acidities exhibit first order kinetics, and at high acidities exhibit second-order acid catalysis.^{23,24} All rearrangements, of course, are first order in the hydrazoaromatic. Thus the rate law for the rearrangement of the hydrazoaromatic is of the form shown in equation 13.

$$\text{rate} = k_2[\text{Hyd}][\text{H}^+] + k_3[\text{Hyd}][\text{H}^+]^2 \quad (13)$$

Some selected values of k_2 and k_3 for substituted hydrazobenzenes are listed in Table 1. The data in Table 1 illustrate that electron-donating o- and p-substituents enhance the rate, and that electron-withdrawing substituents reduce the rate of rearrangement.

Table 1: Rate Constants for the Rearrangements of Some
Hydrazoaromatics (RNHNHR') by the One- and Two-
Proton Reactions.^a

R	R'	$k_2 \times 10^3$ ^b	$k_3 \times 10^3$ ^b
2-ClC ₆ H ₄	2-ClC ₆ H ₄		0.000034
2-FC ₆ H ₄	2-FC ₆ H ₄		0.0027
4-ClC ₆ H ₄	4-ClC ₆ H ₄		0.013
Ph	Ph		1.7
2-CH ₃ C ₆ H ₄	2-CH ₃ C ₆ H ₄	0.21	8.5
3-CH ₃ C ₆ H ₄	3-CH ₃ C ₆ H ₄		17
4-PhC ₆ H ₄	Ph		680
4-CH ₃ C ₆ H ₄	4-CH ₃ C ₆ H ₄		1400
β-naphthyl	Ph	0.50	5.0
α-naphthyl	Ph	20.0	130
β-naphthyl	β-naphthyl	460	
α-naphthyl	β-naphthyl	1000	
α-naphthyl	α-naphthyl	1800	
4-CH ₃ OC ₆ H ₄	Ph	8500	

^a This Table was adapted from a Table given by Cox and Buncel.¹⁶ ^b Rate constants calculated at 0 °C in 60:40 dioxane:water with HClO₄ as the catalyzing acid with k_2 in sec⁻¹M⁻¹ and k_3 in sec⁻¹M⁻².

Theory and Mechanism of Rearrangement

Although much was known about the products and kinetics of acid-catalyzed benzidine rearrangements, a decisive conclusion as to the mechanism of rearrangement was difficult to reach. Based on kinetic studies, however, pathways may be written (Scheme 1). These equations require that the protonations in steps 14 and 16 are fast and reversible, a conclusion which has been developed principally from the solvent-isotope work of Banthorpe, Hughes, and Ingold,²⁵ and that the rearrangements involve the rate-determining scission of the N-N bond, which was later proven by Shine and coworkers.^{26,1-6} The major unsolved question in benzidine rearrangements appeared for a

Scheme 1. Kinetic Pathways for Benzidine Rearrangement

long time to be, therefore, how steps 15 and 17 occurred, that is, how the mono-N-protonated and N,N'-diprotonated molecules underwent intramolecular rearrangement. Several theories of rearrangement have been proposed. Buncl and Cox have listed and discussed five theories of rearrangement:¹⁶ the polar-transition-state theory, the π -

complex theory, the caged-radical mechanism, Lukashevich's mechanism, and the C-protonated-intermediate mechanism. The polar-transition-state theory differs from the others in that it proposes the formation of the skeleton of the product during the rate-determining step, rather than via an intermediate.

The polar-transition-state theory. Banthorpe, Hughes, and Ingold proposed that the polar-transition-state theory²⁴ governs all rearrangements, regardless of their order in acid and the type of products which are formed. This theory requires that N-N bond breaking be concerted with the forming of new bonds (C-C or C-N) in the transition state, in which the structural skeleton of the products is being made. Only deprotonation follows rapidly as shown in Scheme 2.

The proponents of this theory call upon the heterolysis of the N-N bond which follows protonation on nitrogen. In some cases, the structure of the hydrazoarene is such that heterolysis is initiated by monoprotection, and the driving force for the rearrangement is the disposition of a unit of positive charge in one half of the heterolyzing molecule and a pair of electrons in the second half. The two halves of the heterolyzing molecule thereby constitute the so-called polar transition state through which the monoprotected molecule proceeds to the product. In other cases monoprotection is not sufficient to initiate heterolysis of

the N-N bond, and this is achieved with the second protonation. In the view of the theory's proponents, this causes heterolysis of the N-N bond because of the repulsion in charges of the two protonated N atoms. Again, heterolysis leads to a polar transition state via which the diprotonated molecule proceeds on the product.

Scheme 2

This Scheme was adapted from the Scheme shown by Cox and Bunce.¹⁶

The π -complex theory. This theory, originally proposed by Dewar in 1946,²⁷ before many of the facts concerning the benzidine rearrangement were known, has been substantially modified since that time.^{10,28} As the basis of the theory, the π -complex is worthy of consideration because of the intuitively attractive idea that interaction between the π electrons of the benzene rings will help the massive geometric change required of the molecule during the reaction. The theory is summarized in Scheme 3. According to this scheme, a π -complex in which the two rings are held by a π -bond would be formed in the rate-determining step. This π -complex then achieves the orientation of the products by rapid rotation of the two parallel rings. The major difference between the polar-transition-state theory and the π -complex theory is the matter of concertedness.

Scheme 3

The caged-radical theory. This theory requires the homolysis of the N-N bond, which generates either a solvent-caged radical and a cation radical in one-proton rearrangements, or a pair of cation radicals in two-proton rearrangements (equation 18).

Recombination in various ways inside this cage would lead to the observed products. This theory could account well for the observed disproportionation products, but a reaction of this type requires escape of radicals from the cage, and it is very difficult to explain "escaped" radicals with the lack of formation of "crossed" products during the simultaneous rearrangement of different hydrazobenzenes. Also, attempts to detect radicals by e.s.r. spectroscopy,^{29,30} trapping experiments³²⁻³⁴ and the CIDNP technique³² have been wholly unsuccessful. However, Parker and coworkers^{35,36} have provided e.s.r. evidence for the formation of a cation radical during the rearrangement of tetraphenylhydrazine (28) in trifluoroacetic acid or trifluoroacetic acid/dichloromethane at room temperature. Thus cation radicals can be formed, at least for this substrate (Scheme 4), under the non-aqueous conditions used.

It is reasonable that radicals would be more likely to occur under these conditions than under those of aqueous acidic solutions normally used for the reaction.

Scheme 4

Lukashevich's theory. This theory was proposed by Lukashevich^{37,38} and has been refuted critically. The failure to isolate the diacid salt of a hydrazocompound suggested the idea that a transition state like 29 for the rearrangement may be involved. However this theory appears

to be inconsistent with kinetic data and needs no further discussion.

The C-protonated intermediate theory. This theory is based on the assumption that the second protonation in two-proton rearrangements occurs on a ring carbon atom (ipso

position) rather than on nitrogen (see 30).^{39,42} The addition of this proton is believed to be rate determining and to be followed by the rearrangement step. Although some examples of ring-protonated aromatic amines are known, among them 4,4'-diprotonated benzidine,⁴² there is no direct evidence that hydrazobenzene undergoes ring protonation prior to or during rearrangement. All proposals for ring protonation are, in that sense, speculative, and based primarily on the idea that protonation at the second nitrogen atom is improbable. This idea came from the lack of correlation of rates with nitrogen basicities¹⁶ and the inability to detect any N,N'-diprotonated hydrazobenzene. If carbon, rather than nitrogen, is protonated, no correlation would be expected. However rearrangement of hydrazobenzene in D_3O^+ failed to place deuterium at any of the ring position (2,3,5,6) of the product (benzidine).⁴² Also the same reaction was carried out in 75% EtOD/D₂O which was 0.1 N in DCl and no ring deuteration of recovered azobenzene was found after partial conversion.¹ These results indicate that, although one cannot test directly for ipso protonation it, too, does not occur.

Application of Woodward-Hoffmann rules to benzidine rearrangements. Each theory of the benzidine rearrangement which was discussed so far has been applied to all rearrangements, regardless of the type of products which are formed. However, Shine mentioned "if it is felt that acid-catalyzed benzidine rearrangements should follow the Woodward-Hoffmann rules we must conclude that some rearrangements may be concerted while others must go through an intermediate of some sort."¹⁵ This statement has turned out to be true in those cases which have been studied.¹⁻⁶ That is, benzidine, o-benzidine and p-semidine type products are formed via [5,5]-, [3,3]- and [1,5]-sigmatropic shifts, respectively. These are processes which comply with conservation of orbital symmetry, and therefore it is now understandable that the cases studied turned out to be concerted rearrangements. In contrast, diphenylene formation is a [3,5]-sigmatropic rearrangement, formally forbidden by conservation of orbital symmetry from being concerted. This was subsequently confirmed experimentally, although a clear picture of this non-concerted rearrangement is yet to be obtained.

Disproportionation

Disproportionation accompanies most acid-catalyzed rearrangements of hydrazoaromatics. In some cases, disproportionation may be the predominant or sole reaction

pathway. As is shown in Table 2, the extent of disproportionation is dependent on the nature of the substituents in the ring. If there are substituents in the 4- and 4'-position, particularly, a high ratio of disproportionation to rearrangement is observed. The stoichiometry of disproportionation is given in equation 19.

Thus two moles of arylamine and one mole of azoarene are formed in the reaction which consumes two moles of the hydrazoaromatic compound. Kinetically, however, the reaction has been found always to be first order in the hydrazoaromatic species. Furthermore, when disproportionation accompanies rearrangement, each reaction has the same kinetic order in acid, whether this be of first,^{43,44} second,^{7,30,34,45-49} or mixed order.^{34,49,50} Also, intramolecularity was verified for the azobenzene formed from disproportionation. All investigations of the disproportionation reaction, therefore, led to the same conclusion, that an intermediate is formed in a pathway that is kinetically identical with that of rearrangement and that the intermediate then oxidizes a second molecule of hydrazoaromatic in a fast step. The puzzle of disproportionation has been centered on what this intermediate might be. Three possibilities for the intermediate have been proposed: a radical or radical ion, a π -complex, and a quinonoid.

Table 2: Yields of Products in the Acid-Catalyzed Reaction of Hydrazoaromatics Which Produce Appreciable Amounts of Disproportionation.^a

R-C ₆ H ₄ -NHNH-C ₆ H ₄ -R'		products (% of total)				order in acid	ref
R	R'	(2,4')	(2,N')	(N,4')	disp.		
4-MeO	H		55	24	20	1	52
4-NHAc	H	+ ^b	+++ ^b	++ ^b	70	1	52
4-F	4'-F		18.5		80	1-2	30
4-Cl	4'-Cl		22		75	2	7,52
4-Cl	H	19	30	20	31	1,2	7,52
4-Br	4'-Br		30		70	1	52
4-I	4'-I				100	2	52
4-Ph	H	+++ ^b	+++ ^b		38	2	52
4-Ph	4'-Ph		25		75	1.8	49
4-NO ₂	H	+++ ^b	+++ ^b	20	40	2	52

^a This table was adapted mostly from one shown by Banthorpe, O'Sullivan, and Ingold.⁵² ^b The plus signs indicate qualitatively that the product was found chromatographically but not assayed quantitatively.

The role of a π -complex in disproportionation was first proposed for hydrazobenzene by Dewar when it was believed that its rearrangement was kinetically first order in acid (Scheme 5).⁵¹ This idea was then adopted by others for reactions that were second order in acid (Scheme 6).³⁰ In 1967 the interesting proposal was made that the intermediate involved in disproportionation had a quinonoid structure, much like one which leads to rearrangement.⁵² The attractiveness of this proposal was that it could account for the marked incidence of disproportionation among 4,4'-disubstituted hydrazobenzenes. That is, such a 4,4'-bonded quinonoid, unable to form a benzidine easily by loss of the substituents, was readily available for a redox reaction with a second molecule of the hydrazoaromatic (Scheme 7). This p-quinonoid intermediate has been verified by finding substantial carbon and nitrogen KIE for the disproportionation of 4,4'-diiodohydrazobenzene.⁵³

Research Plan

Because heavy-atom kinetic isotope effects had been measured in all of the possible types of benzidine rearrangement except the o-semidine rearrangement, the plan was made to choose a suitable candidate for such a study. Ideally, the substrate needed for studying an o-semidine rearrangement is a 4,4'-disubstituted hydrazobenzene, whose kinetics and products of rearrangement have been

Scheme 5

Scheme 6

Scheme 7

established, and which can be labeled conveniently at the nitrogen, and o- and p-carbon atoms. 4,4'-Dichlorohydrazobenzene was chosen, therefore, from among the few appropriately substituted hydrazobenzenes whose rearrangements were characterized in the literature.

The objective was to measure the carbon and nitrogen kinetic isotope effects for the o-semidine rearrangement of 14 by both scintillation counting and mass spectrometric analysis. For this purpose, the preparations of [$^{15}\text{N}, ^{15}\text{N}'$]14, [$2\text{-}^{14}\text{C}$]14, [$4\text{-}^{14}\text{C}$]14, and [$4,4'\text{-}^{13}\text{C}_2$]14 were planned.

The preparation of [$^{15}\text{N}, ^{15}\text{N}'$]-4-chloroazobenzene ([$^{15}\text{N}, ^{15}\text{N}'$]31) was also planned with the intention of measuring nitrogen KIE in the o-semidine rearrangement of [$^{15}\text{N}, ^{15}\text{N}'$]-4-chlorohydrazobenzene ([$^{15}\text{N}, ^{15}\text{N}'$]27).

CHAPTER II

EXPERIMENTAL SECTION

Material and Equipment

Silica Gel

Baker silica gel for flash chromatography (J. T. Baker Chemical Co., 40 fm) was used for flash column chromatography.

p-Dioxane

p-Dioxane (99%) from Aldrich was distilled over lithium aluminium hydride prior to use.

Product Assay Equipment

Quantitative assay of products was performed with a Waters Associates high-performance liquid chromatograph which was equipped with a Waters Mini Pump (Model 19-60042-055), an Altex analytical UV Detector (Model 153), a Soltec Recorder (Model 252A), and a Varian Integrator (Model CDS 111). A Waters Micro-Bondapak C-18 column was used for separations.

Spectroscopy Equipment

Infrared spectra (IR) were recorded on a Beckman Acculab 8 or Nicolet MX-S FT-IR spectrophotometer. ^1H NMR

spectra were recorded with a Varian EM 360 or EM 360A spectrometer. Chemical shifts are reported in parts per million (ppm) on the scale downfield from internal tetramethylsilane (TMS). Routine mass spectra were taken with a Hewlett-Packard mass spectrometer (Model 5995B), and high resolution mass spectra for assaying isotope enrichments were made in the Midwest Center for Mass Spectrometry at the University of Nebraska, Lincoln.

Equipment for the Measurement of Kinetic Isotope Effects

Whole-molecule-ion mass spectrometry (WMIMS) measurements were made with a Hewlett-Packard quadrupole spectrometer (Model 5995B), operated in the selected-ion-monitored mode. This spectrometer had both externally heated and ordinary internally heated direct insertion probes. Isotope-ratio mass spectrometry was carried out in the laboratory of the Krueger Geochron Co. with use of a VG Micromass Model 903 triple-collector, isotope-ratio spectrometer. Scintillation counting was carried out with a Beckman Model LS 7000 liquid scintillation counter.

Preparation of Compounds Required Routinely

Manganese Dioxide

Preparation of activated manganese dioxide was accomplished according to a literature procedure.⁵⁴ A

solution of manganese sulfate monohydrate (141 g, 0.87 mol) in 250 mL of water and a solution of sodium hydroxide (40%, 195 mL) were added simultaneously over a period of 1 hour to a hot, magnetically stirred, solution of potassium permanganate (160 g, 1.01 mol) in 1 L of water. Manganese dioxide precipitated soon after the start as a fine brown solid. Stirring was continued for 1 hour at 90 °C and the hot suspension was filtered. The manganese dioxide was washed with hot water until the filtrate was free of permanganate and neutral in pH. The product was dried for 4 hours at 125 °C, then ground and redried for 24 hours at 125 °C.

Pyrogallol Solution⁵⁵

Pyrogallol(15 g) was pulverized and dissolved in 100 mL of sodium hydroxide solution. This solution was used to remove oxygen present in cylinder nitrogen.

Dry Sodium Carbonate

Analytical grade sodium carbonate in a weighing bottle was dried in the oven at 105-110 °C for 2 hours. This was used for the standardization of perchloric acid.

Standardization of Perchloric Acid

Well dried sodium carbonate (60 mg) was weighed in a weighing bottle and transferred to a 50 mL Erlenmyer flask with water (10 mL). The solution was titrated with 100-fold

diluted perchloric acid (MCB, 72%) using bromocresol green as an indicator.

Preparation of Labeled 4,4'-
Dichloroazobenzene

The syntheses of labeled 4,4'-dichlorohydrazobenzene are shown for guidance in Schemes 8-11.

Synthesis of [^{15}N , $^{15}\text{N}'$]-
4,4'-Dichloroazobenzene

[^{15}N]-4-Chlorobenzamide (32). A solution of 4.0 g (73.4 mmol) of [^{15}N]ammonium chloride (Stohler, 99% ^{15}N) was made in 36 mL of water. Benzene (3 mL) was layered on top of the solution which was kept at 6 °C. A solution of 6.8 g of sodium hydroxide in 30 mL of water, cooled to 6 °C, was added slowly from a pipette tip which was kept under the surface of the benzene layer. A solution of 12.9 g (73.4 mmol) of 4-chlorobenzoyl chloride in 300 mL of cold benzene was added quickly. The reaction mixture was stirred vigorously for 2 hours at room temperature, during which time the product precipitated. This was filtered to give 11.3 g (72.2 mmol, 98%) of [^{15}N]-4-chlorobenzamide, mp 178-179 °C.

[^{15}N]-4-Chloroaniline ([^{15}N]16). Bromine (13.3 g, 83 mmol) was added dropwise to an ice-cold, stirred solution of 19.4 g of sodium hydroxide in 320 mL of water. After

5 min 11.3 g (72.1 mmol) of [^{15}N]-4-chloroaniline was added and mixture was stirred at 0 °C for 20 min. The mixture was then slowly heated to 95 °C and maintained at that temperature for 1.5 hours. After the reaction mixture was cooled to room temperature, the dark-brown product was filtered, giving 7.8 g of [^{15}N]-4-chloroaniline, mp 60-64 °C. The filtrate was extracted three times with ether, and the extract was dried over sodium sulfate and evaporated to leave 0.5 g of [^{15}N]-4-chloroaniline, mp 65-69 °C. The total yield of crude product was 8.3 g (64.5 mmol, 90%).

[^{15}N , $^{15}\text{N}'$]-4,4'-Dichloroazobenzene ([^{15}N , $^{15}\text{N}'$]₁₇).⁵⁶ A suspension of 3.7 g (28.9 mmol) of [^{15}N]-4-chloroaniline and 20 g of active manganese dioxide in 240 mL of benzene was stirred under reflux for 5 hours in a Dean-Stark apparatus to remove water. The hot solution was filtered and the manganese dioxide was washed with hot benzene. The filtrate was evaporated to give 3.2 g (87%) of [^{15}N , $^{15}\text{N}'$]-4,4'-dichloroazobenzene, mp 170-185 °C. This product was purified by flash column chromatography on silica gel with benzene as eluent, and recrystallized from chloroform giving 2.5 g (10.0 mmol, 70%), mp 188-189 °C. Lit.⁵⁶ mp 185 °C.

Scheme 8

Synthesis of [4,4'-¹³C₂]-4,4'-
Dichloroazobenzene

Sodium nitromalonaldehyde monohydrate (33). In a 1 L Erlenmyer flask, equipped with a thermometer, dropping funnel and magnetic stirrer, were placed sodium nitrite (100 g, 1.45 mol) and water (97 mL). This mixture was heated until all of the sodium nitrite dissolved. A solution of mucobromic acid (100 g, 0.39 mol, Aldrich) in 95% ethanol (97 mL) was added dropwise with vigorous stirring over a period of 1.5 hours. An exothermic reaction occurred and the solution in the flask became deep red and gas was given off. During the addition the temperature was maintained at 54 ± 2 °C. The reaction mixture was stirred for an additional 30 min at this temperature, and cooled to 0-5 °C. A fine yellow precipitate formed which was collected on a Büchner funnel. This crude product was crystallized from 80% ethanol to give 19.7 g (0.13 mol, 33%) of 33. Lit.⁵⁷ yield 36-41%.

[1-¹³C]-4-Nitrophenol (34). This was prepared by method of Swart and Gulik⁵⁸ as follows. To a solution of 10.6 g (69.2 mmol) of sodium nitromalonaldehyde monohydrate and 1.95 g of sodium hydroxide in 230 mL of water cooled to 4 °C was added 3.28 g (55.6 mmol, 99% ¹³C) of [2-¹³C]-acetone (Merck). The mixture was diluted with water to 650 mL, and allowed to stand for 7 days in the refrigerator at 1-2 °C. To the resulting solution cold aqueous sodium

Scheme 9

hydroxide (50%, 318 mL) was added slowly, such that the temperature was maintained at 4-6 °C. The mixture was stirred 2 hours at 5.5 °C, acidified with hydrochloric acid (12 N, about 350 mL) while the temperature was kept below 30 °C, and filtered from a brown solid. For convenience the mixture was divided into three equal portions, and each was extracted with ether (10 x 225 mL). The extracts were dried over sodium sulfate, filtered and evaporated to give 5.02 g (35.9 mmol, 64%) of crude brownish-yellow [1-¹³C]-4-nitrophenol. This was purified with flash column chromatography on silica gel, eluting with a mixture of chloroform and ethanol (100 : 1), giving 4.3 g (30.7 mmol, 55%), mp 109-112 °C. Lit.⁵⁸ mp 108-109 °C.

[4-¹³C]-4-Chloronitrobenzene (35). The general method of Coe et al.⁵⁹ of replacing phenolic OH with Cl was used as follows. Phosphorous pentachloride (6.4 g, 30.7 mmol) was heated for 4 hours at 100 °C with 8.6 g (91.5 mmol) of phenol. The [1-¹³C]-4-nitrophenol (4.3 g, 30.7 mmol) was added to the cooled product, which was then reheated for 4 hours at 100 °C. The crude tetraaryloxyphosphorous monochloride was then decomposed by heating at 200 °C for 20 min. Vacuum distillation removed phenol first, and gave next, 3.3 g (221 mmol, 68%) of [4-¹³C]-4-chloronitrobenzene, mp 81-82.5 °C. Lit.⁵⁹ mp 83 °C.

[4-¹³C]-4-Chloroaniline ([4-¹³C]16).^{34,60} A solution of stannous chloride dihydrate (15.4 g, 68.1 mmol) in

12.6 mL of concentrated hydrochloric acid was stirred while 3.2 g (20.3 mmol) of [4- ^{13}C]-4-chloronitrobenzene was added. Stirring was continued with gentle heating for 20 min, and 8 mL of 95% ethanol was added to give a clear solution. The reaction mixture was refluxed for 1 hour, and, after cooling to room temperature, was made alkaline with 40% sodium hydroxide solution. A slightly-yellow precipitate formed. This was filtered and washed from the filter with ether. Evaporation of the ether solution gave 667 mg of [4- ^{13}C]-4-chloroaniline, mp 64-68 °C. The aqueous, alkaline filtrate was extracted with ether (25 mL x 6). Drying over sodium carbonate and work up of the ether solution gave an additional 1.53 g of product, mp 65-68 °C. Lit.⁶¹ mp 72 °C. Total yield was 2.20 g (17.1 mmol, 84%).

[4,4'- $^{13}\text{C}_2$]-4,4'-Dichloroazobenzene (4,4'- $^{13}\text{C}_2$ 17).

This was prepared in 95% yield by the procedure described above for [^{15}N , $^{15}\text{N}'$]-4,4-dichloroazobenzene, and had mp 180-185 °C. Flash chromatography and recrystallization from chloroform gave mp 188-189 °C.

Synthesis of [^{15}N , $^{15}\text{N}'$]-4-Chloroazobenzene

[^{15}N]-4-Chloronitrosobenzene (36). [^{15}N]-4-

Chloronitrosobenzene was made as described for nitrosomesitylene by Ingold,⁶² but with some modification. Finely-powdered potassium persulfate (22.9 g) was used,

instead of the ammonium salt, in 11.5 mL of concentrated sulfuric acid, and poured onto 10 mL of ice and water. After neutralizing the solution with solid sodium carbonate, 4.0 g (31.1 mmol) of powdered [^{15}N]-4-chloroaniline was added and the mixture was stirred for 1 hour at 5 °C. The solid product was filtered, and washed with water and 1 N hydrochloric acid to give 2.35 g (16.5 mmol, 53%) of crude [^{15}N]-4-chloronitrosobenzene. This product was used directly without steam distillation.

[^{15}N , $^{15}\text{N}'$]-4-Chloroazobenzene ([^{15}N , $^{15}\text{N}'$]31).

Condensation of p-chloroaniline with nitrosobenzene has been described by Jacobson and Loeb.⁶³ The method was adapted as follows. A solution of 1.85 g (13.0 mmol) of [^{15}N]-4-chloronitrosobenzene in 5 mL of acetic acid was added to a solution of 1.22 g (13.0 mmol) of [^{15}N]-aniline (Stohler, 99% ^{15}N) in 3 mL of acetic acid. The mixture was stirred for 1 hour, and allowed to stand for 7 hours at room temperature. The solid product was filtered and dried to give 2.17 g (10.0 mmol, 76%) of [^{15}N , $^{15}\text{N}'$]-4-chloroazobenzene, mp 84-86 °C. A further 0.3 g of product was obtained, mp 70-75 °C, by adding water to filtrate. Recrystallization from ethanol gave mp 87-88 °C. Lit.⁶³ mp 90-91 °C.

Synthesis of [2-¹⁴C]-
4,4'-Dichloroazobenzene

[2-¹⁴C]-Nitrobenzene (37). A solution of 140 mg (4.42 mCi) of [1-¹⁴C]-2-nitroaniline (Hoechst) and 14.85 g (108 mmol) of 2-nitroaniline in 73 mL of 50% hyphosphorous acid which contained a small amount of cuprous oxide was stirred at 0 °C while a solution of 9.4 g (140 mmol) of sodium nitrite in 20 mL of water was added dropwise during 1 hour. The solution was made alkaline with 40% sodium hydroxide and extracted with 4 x 200 mL of ether. The ether extract was dried over sodium sulfate, filtered and evaporated to give dark brown [2-¹⁴C]-nitrobenzene, which was distilled under reduced pressure. The labeled product was washed through the distillation apparatus by adding nitrobenzene to the residue in the distillation flask and distilling through the same apparatus. Finally 20.7 g (65.7%, based on o-nitroaniline used) of [2-¹⁴C]-nitrobenzene was obtained. Assay by scintillation counting showed the product to have an activity of 15.7 mCi/mol.

[2-¹⁴C]-Aniline (38). In a 500 mL, three-neck flask, equipped with a condenser and dropping funnel was placed 14.8 g (120 mmol) of [2-¹⁴C]-nitrobenzene, 51.4 g (508 mmol) of triethylamine, and 300 mg of 10% palladium on charcoal. This mixture was stirred at 90 °C while 21.1 g (463 mmol) of formic acid was added dropwise and then was heated under reflux for 5 hours. After cooling to room temperature, the

Scheme 10

Scheme 11

reaction mixture was diluted with 250 mL of methylene chloride and filtered. The filtrate was dried over magnesium sulfate and evaporated to give crude [2-¹⁴C]-aniline. This crude product was used for acetylation directly without further purification.

[2-¹⁴C]-Acetanilide (39). Into the solution of [2-¹⁴C]-aniline in 15 mL of acetic acid was added dropwise 20 mL of acetic anhydride. This reaction mixture was stirred at room temperature for 24 hours. The resulting solution was poured onto 400 mL of ice water and stirred for 30 min. An almost colorless precipitate formed and was filtered and washed with water. After drying in a desiccator, 10.6 g (79 mmol, 66% based on nitrobenzene) of product was obtained, mp 112-114 °C. Lit.⁶¹ mp 113-114 °C.

[2-¹⁴C]-4-Chloroacetanilide (40). [2-¹⁴C]-4-Chloroacetanilide was prepared from [2-¹⁴C]-acetanilide by some modification of the method of the Orton and Jones.⁶⁴ Chlorox (5% NaOCl, 120 mL, 85 mmol) was slowly run into a solution of 10.6 g (79 mmol) of [2-¹⁴C]-acetanilide in 105 mL of glacial acetic acid. After stirring the mixture for 2 days at room temperature, the colorless precipitate which had formed was collected, washed with water and dried in the air to give 5.6 g (33mmol, 42%) of [2-¹⁴C]-4-chloroaniline, mp 179-180 °C. The product was shown by gc to be free of the o-isomer (a control experiment with unlabeled acetanilide was conducted and the product was analyzed by

gc). The filtrate was diluted with 540 mL of water, giving 980 mg of a tan mixture of labeled o- and p-chloro-acetanilide (1 : 4). This was heated at 75-80 °C under atmospheric pressure to remove the o-isomer by sublimation. The residue was triturated with 25 mL of benzene and crystallized from aqueous acetic acid, giving 450 mg of [2-¹⁴C]-4-chloroacetanilide, mp 179-180 °C. The total yield was 606 mg (45%). Lit.⁶⁴ mp 179 °C.

[2-¹⁴C]-4-Chloroaniline ([2-¹⁴C]16). A mixture of 6.06 g (36 mmol) of [2-¹⁴C]-4-chloroacetanilide in 320 mL of 15% sulfuric acid was refluxed for 5 hours. After cooling, 98 g of sodium carbonate was added slowly to the solution and the alkaline solution was extracted with ether (9 x 100 mL). The ether solution was dried over magnesium sulfate, filtered and evaporated to give 4.18 g (92%) of [2-¹⁴C]-4-chloroaniline, mp 68-69 °C. Lit.⁶¹ mp 72 °C. Assay by scintillation counting showed this to have an activity of 16.7 mCi/mol. This product was mixed with 14.0 g of p-chloroaniline to give a calculated activity of 3.8 mCi/mol. The mixture was used for preparation of [2-¹⁴C]-4,4'-dichloroazobenzene.

[2-¹⁴C]-4,4'-Dichloroazobenzene ([2-¹⁴C]17). This was prepared by the procedure described earlier for [¹⁵N,¹⁵N']-4,4'-dichloroazobenzene. The yield was 16.11 g (90%) and recrystallization from chloroform gave mp 188-189 °C. Lit.⁵⁶ mp 185 °C.

Synthesis of [4-¹⁴C]-
4,4'-Dichloroazobenzene

[4-¹⁴C]-4-Chloronitrobenzene (41). A mixture of 37 mg (1.55 mCi) of [1-¹⁴C]-4-nitroaniline (Hoechst) and 3.47 g (25 mmol) of p-nitroaniline was dissolved in 7.0 mL of concentrated hydrochloric acid. To this was added 7.0 mL of water and to this solution, cooled to 0 °C, was added slowly a pre-cooled solution of 1.9 g (28 mmol) of sodium nitrite in 4.0 mL of water. The mixture was stirred vigorously for complete solution of solid and poured slowly into a pre-cooled solution of 3.5 g (35 mmol) of cuprous chloride in 14 mL of concentrated hydrochloric acid. This mixture was allowed to room temperature and stirred until evolution of nitrogen gas ceased. Steam distillation gave 3.6 g (23 mmol, 90%) of [4-¹⁴C]-4-chloronitrobenzene, mp 81-82.5 °C. Lit.⁶¹ mp 83 °C.

[4-¹⁴C]-4-Chloroaniline ([4-¹⁴C]16). [4-¹⁴C]-4-Chloronitrobenzene (3.6 g) was reduced with stannous chloride dihydrate and hydrochloric acid to form [4-¹⁴C]-4-chloroaniline in 90% yield by the procedure described earlier for [4-¹³C]-4-chloroaniline, and had mp 68-69 °C. Assay showed the product to have an activity of 60 mCi/mol. A 2.66 g portion of the product was diluted with p-chloroaniline and the mixture was crystallized from methylene chloride and petroleum ether so that the final product, [4-¹⁴C]-4-chloroaniline, had an activity of 4.2 mCi/mol.

[4-¹⁴C]-4,4'-Dichloroazobenzene ([4-¹⁴C]17). The recrystallized [4-¹⁴C]-4-chloroaniline (20.0 g, 156 mmol) was diluted once again with 10.0 g (78.4 mmol) of 4-chloroaniline. This mixture was oxidized with manganese dioxide using the procedure described earlier to give 27.5 g (110 mmol, 93%), mp 187-189 °C. Recrystallization from chloroform gave 25.6 g, mp 188-189 °C. Lit.⁵⁶ mp 185 °C.

Preparation of Unlabeled Products

Preparation of p-Chloroacetanilide (41)

p-Chloroacetanilide was prepared by a general method for acetylation of aromatic amines as follows. A solution of 0.54 g (4.20 mmol) of p-chloroaniline in 7 mL of acetic acid and 0.5 mL (5.3 mmol) of acetic anhydride was refluxed for 5 hours. The resulting solution was cooled and poured onto 70 mL of ice water. The colorless precipitate was filtered and washed with cold water to obtain 0.66 g (93%) of p-chloroacetanilide, mp 179-181 °C (after crystallization from acetic acid). Lit.⁶¹ mp 179 °C. ¹H NMR (acetone-d₆) δ 10.08 (s, br, 1H), 7.20-7.70 (d of d, 4H), and 2.1 (s, 3H).

Preparation of 4-Chloro-N-trifluoroacetylaniline (20)⁶⁵

p-Chloroaniline (1.0 g, 7.0 mmol) was dissolved in 25 mL of benzene. To this 1.7 g (7.9 mmol) of trifluoroacetic anhydride (TFA) was added dropwise with continuous stirring at room temperature. After 30 min, the colorless

precipitate which was formed was filtered. A second crop of crystals was obtained by evaporation of the mother liquor. The total yield was 1.74 g (98%), mp 123.5-124.56 °C (benzene). Lit.⁶⁵ mp 123-124 °C (hexane-acetone). ¹H NMR (acetone-d₆) δ 10.3 (s, br, 1H) and 7.30-7.90 (d of d, 4H).

Synthesis of 2'-Amino-4,5'-dichlorodiphenylamine (15)

4,5'-Dichloro-2'-nitrodiphenylamine (42). Following the method of Kottenhahn et al.,⁶⁶ a solution of 20.3 g (0.1 mol) of 1-chloro-3,4-dinitrobenzene (Aldrich) and 41.5 g (0.3 mol) of p-chloroaniline in 150 mL of ethanol was kept at 40-50 °C for 12 hours. The orange product which formed on cooling the solution was filtered and washed with cold ethanol and 3 N hydrochloric acid, giving 13.7 g (48 mmol, 48%) of 4,5'-dichloro-2'-nitrodiphenylamine, mp 157-158 °C. Lit.⁶⁶ mp 158-158.5 °C. ¹H NMR (CDCl₃) δ 9.45 (s, br, 1H), 8.15 (d, 1H), 7.30-7.50 (m, 5H), and 6,7 (d of d, 1H).

2'-Amino-4,5'-dichlorodiphenylamine (15). To a solution of 1.0 g (3.5 mmol) of 4,5'-dichloro-2-nitrodiphenylamine in 5 mL of 95% ethanol was added 2.7 g of tin shot and 9 mL of concentrated hydrochloric acid. The reaction mixture was refluxed for 2 hours to give a clear, greenish solution. The solution was cooled to room temperature to precipitate a colorless solid, which was filtered and kept. The filtrate

was poured onto 40 mL of ice water, and a second crop of product was obtained. This was combined with the previous crop and dissolved in 20 mL of ethanol. After addition of 20 mL of 40% aqueous sodium hydroxide, the mixture was stirred for 20 min and extracted with ether (3 x 300 mL). The ether extract was dried over sodium carbonate, filtered and evaporated to obtain 0.43 g (1.7 mmol, 49 %) of 2'-amino-4,5'-dichlorodiphenylamine, mp 89-91 °C (acetone-water). Lit.⁷ mp 90 °C. ¹H NMR (CDCl₃) δ 6.65-7.30 (m, 7H), 3.75 (br, 3H).

Acetylation of 2'-Amino-4,5'-dichlorodiphenylamine (15)

A solution of 0.1 g (0.4 mmol) of 15 in 2 mL (21 mmol) of acetic anhydride was stirred at room temperature for 1 hour. A colorless precipitate formed, which was filtered and washed with water. The filtrate was poured onto 20 mL of ice water. This mixture was stirred for a while and the precipitate which had formed was filtered and washed with water. The product obtained in almost quantitative yield (0.12 g, 0.4 mmol) was 2'-acetylamino-4,5'-dichlorodiphenylamine (43), mp 188.5-190 °C (methylene chloride-petroleum ether). ¹H NMR (acetone-d₆) δ 9.40 (s, br, 1H), 7.68 (s, br, 1H), 6.65-7.30 (m, 7H), and 2.07 (s, 3H). IR (KBr) 3280 (s), 3370 (m), 1630 (s), and 1580 (s) cm⁻¹. Mass spectrum, m/e 294.10 (M⁺, 83%).

Acetylation of 15 under refluxing conditions was found to give a different product. A solution of 0.12 g (0.48 mmol) of 15 in 0.1 mL of acetic anhydride and 3 mL of acetic acid was refluxed for 2 hours. After cooling, the solution was poured onto 50 mL of ice water and stirred for a while. A precipitate did not form. Therefore the solution was extracted with ether (4 x 20 mL) and the extract was washed with water, dried over potassium carbonate, filtered and evaporated to afford 0.13 g (98%) of a product having mp 131-132 °C (ethanol-water). The product was identified as the 44 by mass, IR and ^1H NMR spectroscopy. ^1H NMR (acetone- d_6) δ 7.12-7.8 (m, 7H), 2.4 (s, 3H); IR (KBr) 3000 (m), 1600 (m), 1580 (m), and 1480 (s) cm^{-1} , there were no peaks in the NH and C=O regions ; mass spectrum, m/e 276.10 (M^+ , 100%).

Trifluoroacetylation of 2'-Amino-4,5'-dichlorodiphenylamine

Reaction of 15 with trifluoroacetic acid failed to give the trifluoroacetyl derivative even when carried out at -6 °C. The product always contained some diazole (19). Therefore, the formation of 19 alone was sought and was achieved by boiling a solution of 93 g (0.37 mmol) of 15 in 5 mL of benzene containing 0.2 mL (1.33 mmol) of TFA. Evaporation of the solvent gave 120 mg (0.36 mmol, 98%) of 19, mp 95-96 °C (petroleum ether, bp 30-60 °C). ^1H NMR

(acetone- d_6) δ 7.32-7.97 (m). There were no peaks in the NH and C=O regions of the infrared spectrum (KBr). High resolution mass spectrum, M^+ (46.5%) 329.9955. Calcd for $C_{14}H_7N_2F_3Cl_2$: 330.0021.

Bis(trifluoroacetyl) derivative of the p-semidine.

Attempts to prepare the authentic p-semidine (4-amino-4'-chlorodiphenylamine, 18) were not successful. Therefore, the identity of 21 (bis(trifluoroacetyl) derivative of p-semidine) was established after its isolation from the rearrangement of unenriched 4,4'-dichlorohydrazobenzene. Product 21 had mp 121-122 °C (methylene chloride-petroleum ether); IR 3304 (s), 1722 (s). 1695 (s) cm^{-1} , mass, m/e 410.2 (M^+ , 100 %). For the KIE studies, trifluoroacetylation of the mixture of amine products was carried out to ensure that none of the monotrifluoroacetyl derivative of the p-semidine remained. When the conditions of trifluoroacetylation were mild (1 hour of refluxing rather than 2 hours of refluxing and standing 24 hours at room temperature), the monotrifluoroacetyl derivative of p-semidine was isolated after workup of the rearrangement

products, and had mp 178-179 °C (chloroform); IR 3366 (s) and 1697 (s) cm^{-1} . High resolution mass spectrum, M^+ (100%) 314.0438. Calcd for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{OF}_3\text{Cl}$: 314.0521.

Trifluoroacetylation of Diphenylamine

The solution of 1.67 g (9.9 mmol) of diphenylamine in 3 mL of benzene was stirred at room temperature for 5 hours. More benzene (20 mL) was then added, and the solution was washed twice with saturated sodium bicarbonate solution (20 mL) and twice with water. The benzene solution was dried over sodium sulfate, filtered and evaporated to leave 2.43 g of N-trifluoroacetyldiphenylamine. The yield was 93%, mp 70-71 °C (methylene chloride-petroleum ether): ^1H NMR (chloroform-d) δ 7.3 (s); IR (KBr) 3067 (m), 1716 (s), 1591 (s), and 1491 (s); mass spectrum, m/e 265 (M^+ , 100%).

Preparation of Mixtures of Labeled and Unlabeled 4,4'-Dichloroazobenzene

5 Mol % of [^{15}N , $^{15}\text{N}'$]-4,4'-Dichloro-azobenzene in 4,4'-Dichloroazobenzene

A mixture of [^{15}N , $^{15}\text{N}'$]17 (1.0 g, 3.95 mmol) and unlabeled 17 (19 g, 75.70 mmol) was recrystallized from

chloroform to give orange-red crystals, 18.6 g (93%), mp 188-189 °C. Lit.⁵⁶ mp of unlabeled 17 185 °C.

5 Mol % of [4,4'-¹³C₂]-4,4'-Dichloro-azobenzene in 4,4'-dichloroazobenzene

A mixture of [4,4'-¹³C₂]-17 (1.0 g, 3.95 mmol) and unlabeled 17 (19 g, 75.7 mmol) was recrystallized from chloroform to give orange-red crystals, 18.5 g (93%), mp 188-189 °C.

These mixtures were used to prepare labeled 4,4'-dichlorohydrazobenzene.

Preparation of Labeled 4,4'-Dichlorohydrazobenzene

4,4'-Dichlorohydrazobenzene (14) was prepared immediately before its use.

5 Mol % of [¹⁵N, ¹⁵N']-4,4'-Dichlorohydrazobenzene

Diluted [¹⁵N, ¹⁵N']17 (6.0 g, 23.9 mmol) in 120 mL of acetone was stirred with 8.0 g of zinc dust. A saturated solution of aqueous ammonium chloride was added until the yellow color of 17 had disappeared completely. The colorless solution was filtered through fritted glass into 400 mL of 10% ammonium hydroxide solution which had been degassed. The suspension of 14 was stirred for coagulation under aspirator vacuum for 30 min. The colorless 14 was filtered,

washed with water and dried under vacuum, giving 5.9 g (98%), mp 125-126 °C. Lit.⁷ mp 120 °C. Unlabeled 4,4'-dichlorohydrazobenzene (14) had the following spectral data. ¹H NMR (chloroform-d) δ 7.20-6.60 (d of d, 8H) and 5.4 (s, br, 2H).

5 Mol % of [4,4'-¹³C₂]-4,4'-
Dichlorohydrazobenzene

The same procedure as above was applied giving similar results.

5 Mol % of [4-¹⁴C]-4,4'-
Dichlorohydrazobenzene

The same procedure as above was applied giving similar results.

5 Mol % of [2-¹⁴C]-4,4'-
Dichlorohydrazobenzene

The same procedure as above was applied giving similar results.

Benzidine Rearrangement

General Conditions for the Rearrangement of 4,4'-Dichlorohydrazobenzene

Acid catalyzed rearrangements of 4,4'-dichlorohydrazobenzene (14) were carried out at 0 °C in 60% aqueous dioxane (60 : 40 dioxane-water) to verify the kinetic conditions and rate constants of earlier work.⁷ In each run the ionic strength of the solution was maintained at 1.0 by adding the required amount of lithium perchlorate trihydrate. The starting concentrations of 14 and perchloric acid were 0.02-0.03 M and 0.5 M respectively.

Rearrangement of [¹⁵N, ¹⁵N']-4,4'-Dichlorohydrazobenzene ([¹⁵N, ¹⁵N']14)

An example of the general procedure is given. A solution of 5.06 g (20 mmol) of [¹⁵N, ¹⁵N]-4,4'-dichlorohydrazobenzene, which contained 5 mol % of the labeled isomer, in 300 mL of dioxane and 200 mL of water was cooled to 0 °C. A second solution, also cooled to 0 °C, was made by dissolving 42.8 mL (0.50 mol) of standardized 72% perchloric acid and 80.2 g (0.50 mol) of LiClO₄·3H₂O in 300 mL of dioxane, and dilution to 500 mL with water. The pre-cooled solutions were flushed with argon, mixed quickly and kept under argon. After 5 min a 200 mL aliquot was pumped out under argon pressure and kept in the refrigerator under argon for 48 hours.

Low Conversion. After 30 min from mixing, the remaining cold solution (800 mL) was filtered by suction through sintered glass into a flask containing 17 g (0.425 mol) of NaOH in 50 mL of water. The acid solution was kept cold during filtration, which took 6 min. The filtered solid was washed with 60% aqueous dioxane and dried, giving 290 mg (1.16 mmol, 7.2%) of 4,4'-dichloroazobenzene (17). Air was bubbled gently into the alkaline solution for 36 hours, after which 17 (3.1 g, 12.4 mmol, 77.5%) was again recovered. The filtrate was extracted with 9 x 100 mL portions of ether, and the ether solution was dried and most of the solvent was evaporated. To the residue was added 2 mL of benzene and 1 mL of TFA. The mixture was refluxed for 2 hours, and allowed to stand for 24 hours. Next, 20 mL of benzene was added and the solution was washed twice with 30 mL of saturated NaHCO_3 solution, twice with water and then dried over sodium sulfate. Evaporation gave 930 mg of mixed derivatives which were separated into the following components by flash column chromatography, using benzene elution, and collecting 20-mL aliquots: 54 mg (0.22 mmol, 1.3%) of 17 (fractions 1-4); 109 mg (0.33 mmol, 2.1%) of o-semidine derivative, 19 (fractions 8-11); 380 mg (1.70 mmol, 5.3%) of the trifluoroacetyl derivative of p-chloro-aniline, 20 (fractions 14-24); and 84 mg (approx 1.5%) of a mixture of 19 and 20. Finally, elution with methylene chloride gave 122 mg (0.30 mmol, 1.9%) of 21 the

bis(trifluoroacetyl) derivative of the p-semidine (fraction 27-29). Each product was recrystallized three times for mass spectrometry giving: 20 (benzene) 156 mg, mp 123-124 °C; 19 (petroleum ether), 13.5 g, mp 95-96 °C; 21 (CH₂Cl₂-petroleum ether) 50 g, mp 121-122 °C.

100% Conversion. After standing for 24 hours, the 200 mL aliquot was filtered, as earlier, into a solution of 4.5 g (0.114 mol) of sodium hydroxide in 15 mL of water, giving 400 mg (1.59 mmol, 39.7%) of 17. The filtrate was treated exactly as before but without prior air bubbling. Flash chromatography gave: 19 mg (0.08 mmol, 1.8%) of 17 (fractions 1-4); 92 mg (0.28 mmol, 6.8%) of 19 (fractions 8-10); 500 mg (2.24 mol, 27.3%) of 20 (fractions 13-27); and 83 mg (approx 5.6%) of a mixture of 19 and 20. Elution with methylene chloride gave 221 mg (0.54 mmol, 13.1%) of 21 (fractions 33-40). Crystallization followed as above.

This experiment was repeated three times in all. Product distributions are summarized in Table 3.

Rearrangement of [4,4'-¹³C₂]-4,4'-
Dichlorohydrobenzene ([4,4'-¹³C₂]14)

Rearrangement of [4,4'-¹³C₂]-4,4'-dichlorohydrobenzene were carried out in the same way. Times for low conversions were 24, 32, and 38 min, while initial concentrations of [4,4'-¹³C₂]14 were correspondingly 0.028, 0.02, and 0.02 M. After trifluoroacetylation, products were

Table 3: Yields^a of Products in the Acid-Catalyzed
Reaction of 4,4'-Dichlorohydrazobenzene (14).

run	isotope enrich. ^b	% conv. ^c	% <u>20</u>	% <u>19</u>	% <u>21</u>	% <u>17</u> A ^d B ^e	total recov, %
1	¹⁵ N	22.2	6.1	2.3	1.8	7.8 77.8	95.8
		100	30.3	10.9	9.2	38.4	88.8
2	¹⁵ N	22.5	5.9	2.9	1.9	8.5 77.5	96.7
		100	30.2	10.0	13.4	41.6	95.2
3	¹⁵ N	20.0	5.8	1.9	1.9	6.8 80.0	96.4
		100	28.3	10.2	13.4	40.1	92.2
4	¹³ C	20.2	5.8	2.5	1.3	8.5 80.0	98.1
		100	29.9	11.0	13.0	42.3	98.6
5	¹³ C	24.3	7.4	2.9	2.4	8.2 75.7	96.6
		100	30.8	10.0	13.3	41.7	95.8
6	¹³ C	14.7	4.2	1.8	1.0	6.1 85.3	98.4
		100	30.7	10.8	12.7	42.5	96.7
13 ^f	none	100	26.3	14.8	12.1	40.3	93.5

^a Expressed as % of 14 converted into product. ^b Two atoms.

^c Based on the amount of 17 recovered after air oxidation of unreacted 14. ^d Filtered from acid solution. ^e Recovered

after air oxidation of unreacted 14. ^f Assays by HPLC. All other runs by flash chromatography.

separated by flash column chromatography and each product was recrystallized three times for mass spectrometry. Product distributions are summarized in Table 3.

Rearrangement of [4-¹⁴C]-4,4'-
Dichlorohydrazobenzene ([4-¹⁴C]14)

Rearrangements of [4-¹⁴C]-4,4'-dichlorohydrazobenzene were carried out in the same way. Times for low conversion were 28, 33, and 25 min, while initial concentration of [4-¹⁴C]14 was 0,026 M. Products were separated by flash chromatography after trifluoroacetylation. Each trifluoroacetylated product was sublimed once after three recrystallizations and prior to scintillation counting.

Rearrangement of [2-¹⁴C]-4,4'-
Dichlorohydrazobenzene ([2-¹⁴C]14)

Rearrangements of [2-¹⁴C]-4,4'-dichlorohydrazobenzene were carried out in the same way. Times for low conversions were 32.5, 34, and 28 min, while the initial concentration of 14 was 0.019 M. Only o-semidine and p-chloroaniline derivatives, 19 and 20, were separated by flash chromatography. Each trifluoroacetylated product was sublimed once after three recrystallizations and prior to scintillation counting.

KIE Measurements

Whole-molecule-ion mass spectrometry (WMIMS) was successfully applied only in measuring the isotopic abundances of products 20 and 21 from rearrangement of [$^{15}\text{N},^{15}\text{N}'$]14. Reproducible results could not be obtained by WMIMS for [$^{15}\text{N},^{15}\text{N}'$]19, or for any of the derivatives of the ^{13}C -labeled products. Failure to obtain reproducible results with samples of 19 derived from the difficulty in producing a steady concentration of 19 in the mass spectrometer, and this led to problems with the electron multiplier. Failure to obtain reproducible results with any ^{13}C -labeled sample derived, as was found later, from the absence of carbon KIE in the rearrangement, resulting in WMIMS measurements which were scattered widely around the error limit of the instrumentation.

WMIMS measurements were made with a Hewlett-Packard quadrupole mass spectrometer (Model 5995B) operated in the selected-ion-monitoring mode. Samples were introduced via a direct insertion probe (DIP) and the source pressure was maintained at 3×10^{-7} torr by controlled heating of the samples. Samples of 20 were heated externally with the specially designed probe shown in Figure 1, and bled into the source via needle valve. A total of 10,000-29,000 scans of relative abundances was made per sample with an average dwell times of 150 ms/ion. The resulting data were analyzed statistically in 25 blocks. That is, for example, when

Figure 1. Direct insertion probe (DIP) for mass spectrometry: (a), normal DIP, (b), modified (DIP).

10,000 scans were made each block of 400 scans was averaged and the 25 results were treated statistically.

Isotope-ratio mass spectrometry was carried out in the laboratory of the Krueger Geochron Co. Samples were mixed with preheated cupric oxide, sealed in an evacuated tube, and heated at 550 °C for 8 hours to convert the samples into a mixture of nitrogen, carbon dioxide, and water. The gases were analyzed for $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios in a triple-collector instrument after cryogenic separation. The method has been described by Sofer.⁶⁷

Scintillation counting was carried with a Beckman Model LS 7000 liquid scintillation counter. Four separate 5 mg samples of each derivative, weighed precisely on a Cahn balance to ± 0.001 mg, were dissolved in 10 mL of cocktail for liquid scintillation counting (SCINT-O, Packard) and counted 7-11 times each. The average of counts per 1.000 mg was used in KIE calculations.

Product Analysis by High-Performance Liquid Chromatography

The products of rearrangement of 4,4'-dichlorohydrazobenzene were analyzed as their trifluoroacetyl derivatives with the use of HPLC. The eluent was 38% aqueous acetonitrile. Quantitative assay were made with a Varian integrating computer (Model-CDS 111) using response factors established with authentic samples. In the assay of

p-chloro-N-trifluoroacetylaniline (20), a calibration curve was also used to check the response factor data. Results are given for run 13 in Table 3.

Preparation of the internal standard solution.

Acetanilide (0.6114 g, 4.53 mmol) was placed in a 50-mL volumetric flask which was filled to the mark with acetonitrile.

Preparation of the standard mixture solution. One mL of acetanilide stock solution (0.0906 mmol), 6.0 mg (0.0181 mmol) of 19, 6.5 mg (0.0159 mmol) of 21, and 11.5 mg (0.0515 mmol) of 20 were placed in a 10-mL volumetric flask which was filled to the mark with acetonitrile. Several 20- μ L aliquots were injected into the HPLC instrument for analysis.

Preparation of a solution of the product mixture for analysis. Rearrangement of 4,4'-dichlorohydrazobenzene (0.25 g, 0.99 mmol) was carried out in the same way as described earlier. After trifluoroacetylation, all of the reaction products and 10 mL of acetanilide stock solution were placed in a 100-mL volumetric flask which was then filled to the mark with acetonitrile. Several 20- μ L aliquots were injected into the HPLC instrument for analysis.

Preparation of Standard Solutions for Calibration Plots

A set of four solutions of authentic 20 was prepared, each containing a different concentration of the authentic compound and a fixed concentration of an internal standard, according to the following method. First, two stock solutions, one (solution A) of 20 (0.302 g in a 100-mL volumetric flask) and the second (solution B) of acetanilide (0.967 g in a 100-mL volumetric flask), were prepared separately in acetonitrile. Then four 25-mL volumetric flasks were made ready. To the flasks was added respectively 1, 2, 3, and 4 mL of solution A and 10 mL of solution B. To each flask acetonitrile was added to the mark. Several 20- μ L aliquots of each sample solution were injected into the HPLC instrument for analysis.

Calibration Plot. A calibration plot was prepared by measuring the integration ratios of the authentic compound and the internal standard from the series of chromatograms and plotting these ratios with respect to the corresponding concentration ratios. A linear calibration plot for 20 was obtained in the concentration range of $1.73\text{--}6.92 \times 10^{-3}\text{M}$.

The relative response factors (F) are listed in Table 4.

Table 4: Relative Response Factors (F).

Authentic Compound (AC)	[AC] x 10 ³ M	[IS] ^a x 10 ³ M	F
p-chloroaniline derivative (<u>20</u>)	5.145	9.060	0.4913
	1.73-6.92 ^b	8.960 ^b	0.5039 ^b
o-semidine derivative (<u>19</u>)	1.810	9.060	0.7258
p-semidine derivative (<u>21</u>)	1.585	9.060	0.2966

^a Acetanilide was used as the internal standard. ^b Data from calibration plot.

CHAPTER III

RESULTS AND DISCUSSION

Syntheses of Labeled Substrates

The objective of the syntheses was to prepare appropriately labeled azoarenes, since these could be reduced easily as needed to labeled hydrazoarenes which could then be used in the kinetic studies. In this work, four of the heavy-atom isotopic derivatives of 4,4'-dichloroazobenzene and one of 4-chloroazobenzene were prepared. These were, [^{15}N , $^{15}\text{N}'$]-, [4,4'- $^{13}\text{C}_2$]-, [4- ^{14}C]-, and [2- ^{14}C]-4,4'-dichloroazobenzene, and [^{15}N , $^{15}\text{N}'$]-4-chloroazobenzene.

[^{15}N , $^{15}\text{N}'$]-4,4'-Dichloroazobenzene ([^{15}N , $^{15}\text{N}'$]17)

Synthesis of [^{15}N , $^{15}\text{N}'$]-4,4'-dichloroazobenzene was carried out as shown in Scheme 8. [^{15}N]-4-chlorobenzamide was prepared from 4-chlorobenzoyl chloride and [^{15}N]-ammonium chloride (99% ^{15}N) in almost quantitative yield. [^{15}N]-4-chloroaniline was obtained by Hofmann rearrangement of [^{15}N]-4-chlorobenzamide and was oxidized to [^{15}N , $^{15}\text{N}'$]-4,4'-dichloroazobenzene with manganese dioxide. The overall yield was 77%.

$[^{15}\text{N}, ^{15}\text{N}']\text{-4-Chloroazobenzene } ([^{15}\text{N}, ^{15}\text{N}']\underline{31})$

Synthesis of $[^{15}\text{N}, ^{15}\text{N}']\text{-4-chloroazobenzene}$ was carried out as shown in Scheme 8. $[^{15}\text{N}]\text{-4-chloronitrosobenzene}$ was prepared in 53% yield by oxidation of $[^{15}\text{N}]\text{-4-chloroaniline}$ with Caro's acid (potassium persulfate in sulfuric acid). Condensation of $[^{15}\text{N}]\text{-4-chloronitrosobenzene}$ with $[^{15}\text{N}]\text{-aniline}$, which was commercially available, was carried out in acetic acid, following the method of Jacobson and Loeb.⁶³ The overall yield was 36%.

$[4,4'\text{-}^{13}\text{C}_2]\text{-4,4'-Dichloro-azobenzene } ([^{15}\text{N}, ^{15}\text{N}']\underline{17})$

Synthesis of $[4,4'\text{-}^{13}\text{C}_2]\text{-4,4'-dichloroazobenzene}$ was carried out as shown in Scheme 9. Preparation of $[1\text{-}^{13}\text{C}]\text{-4-nitrophenol}$ is well documented and the reported yields vary from 64% to 74%.^{58,68} In this work $[1\text{-}^{13}\text{C}]\text{-4-nitrophenol}$ was obtained in 64% yield in crude form, and in 55% yield after purification by flash chromatography. Conversion of 4-nitrophenol into 4-chloroaniline in two different ways was considered. The first, which was never tried, is shown in Scheme 12. The first four steps of Scheme 12 have been well documented in the synthesis of $[5\text{-}^{14}\text{C}]\text{-guaiacol}$ by Kratzl and Vierhapper,⁶⁹ and showed almost quantitative yields in each step. The main functional group transformation in this plan was the chlorination of acetanilide. This chlorination yields always a mixture of o- and p-isomers and the maximum

Scheme 12

yield of the p-isomer has been reported to be 68% from the use of chlorine or chloramine T.^{70,71} After separation from the o-isomer, 4-chloroacetanilide can be hydrolyzed to 4-chloroaniline in good yield. The overall yield was calculated to be about 57% based on the reported yield in each step. The second way, the one which was carried out, is shown in Scheme 9. In this method, 4-nitrophenol was directly converted to 4-chlorophenol then reduced to 4-chloroaniline. In the conversion of 4-nitrophenol to 4-chloronitrobenzene, phosphorous pentachloride first was treated with 3 mol of phenol to give triphenoxyphosphorus dichloride, which was then treated with 1 mol of p-nitrophenol in situ. The resulting tetraaryloxyphosphorous monochloride was subsequently decomposed to the desired p-chloronitrobenzene. The [4-¹³C]-p-chloronitrobenzene was reduced to [4-¹³C]-p-chloroaniline with stannous chloride and HCl and then oxidized to [4,4'-¹³C₂]-4,4'-dichloroazobenzene.

Isotopic enrichments were determined by whole-molecule-ion mass spectrometry. That is, enrichment in the (M + 1) or (M + 2) molecular ion was measured by comparing the M/(M + 1) or M/(M + 2) abundance in the labeled compounds with that in the corresponding natural compounds. The results are summarized in Table 5. These results show that the projected syntheses were achieved highly successfully. Thus, [¹⁵N, ¹⁵N']-4-chloroazobenzene ([¹⁵N, ¹⁵N']31) and

[^{15}N , $^{15}\text{N}'$]-4,4'-dichloroazobenzene ($([^{15}\text{N}$, $^{15}\text{N}']\underline{17})$) showed 98.6% and 99.1% $^{15}\text{N}_2$ enrichment, respectively, and [$4\text{-}^{13}\text{C}$]-4-chloro-N-trifluoroacetylaniline ($[4\text{-}^{13}\text{C}]\underline{20}$) showed 98.2% ^{13}C enrichment. On the basis of this ^{13}C analysis the enrichment in [$4,4'\text{-}^{13}\text{C}_2$]-4,4'-dichloroazobenzene should have been 96.5% $^{13}\text{C}_2$, 3.5% $^{13}\text{C}_1$, whereas direct analysis gave 95.4% $^{13}\text{C}_2$ and 4.1% $^{13}\text{C}_1$, in very close agreement with the anticipated result.

Table 5: Mass Spectrometric Analysis of the Labeled Substrates.

substrate	composition %			
	$^{15}\text{N}_2$	^{15}N	$^{13}\text{C}_2$	^{13}C
[^{15}N , $^{15}\text{N}'$] <u>31</u>	98.6	1.0		
[^{15}N , $^{15}\text{N}'$] <u>17</u>	99.1	0.49		
[$4\text{-}^{13}\text{C}$] <u>20</u>				98.2
[$4,4'\text{-}^{13}\text{C}_2$] <u>17</u>			95.4	4.1
[$4,4'\text{-}^{13}\text{C}_2$] <u>17</u>			96.5 ^a	3.5 ^a

^a Calculated value based on the ^{13}C analysis of the [$4\text{-}^{13}\text{C}$]-4-chloro-N-trifluoroacetylaniline ($[4\text{-}^{14}\text{C}]\underline{20}$).

[$4\text{-}^{14}\text{C}$]-4,4'-Dichloroazobenzene ($[4\text{-}^{14}\text{C}]\underline{17}$)

Synthesis of [$4\text{-}^{14}\text{C}$]-4,4'-dichloroazobenzene was carried out as shown Scheme 10. Commercially available

[1-¹⁴C]-4-nitroaniline was diazotized and the diazonium salt solution was then treated with cuprous chloride in hydrochloric acid to give [4-¹⁴C]-4-chloronitrobenzene. This was reduced with stannous chloride and hydrochloric acid to [4-¹⁴C]-p-chloroaniline which was next diluted with p-chloroaniline to a calculated radioactivity of 8.4 mCi/mol, and oxidized to [4-¹⁴C]-4,4'-dichloroazobenzene.

[2-¹⁴C]-4,4'-Dichloroazobenzene ([2-¹⁴C]17)

Synthesis of [2-¹⁴C]-4,4'-dichloroazobenzene was carried out as shown Scheme 11. Commercially available [1-¹⁴C]-2-nitroaniline was deaminated via the diazonium salt. The resulting nitrobenzene was reduced to aniline with trimethylamine and palladium on charcoal and the aniline was acetylated before chlorination of the ring. As described earlier, chlorination of acetanilide always gives a mixture of o- and p-isomers. Sodium hypochlorite was chosen from among many chlorinating reagents, because 42-48% of pure p-chloroacetanilide was obtained in many control experiments simply by collecting the product after the reaction. In each control reaction, p-chloroacetanilide which precipitated was analyzed by GC and it was confirmed that there was no o-isomer present. In the reaction of [2-¹⁴C]-acetanilide with sodium hypochlorite, 42% of the pure p-isomer was obtained as a precipitate and another 3% was obtained by workup of the mother liquor and separation from

the o-isomer by sublimation and recrystallization. In 1931 Stephen and coworkers⁷² had reported that the reduction of an aromatic nitro compound with the use of stannous chloride in acetic anhydride provided a simple one-pot means of reducing the nitro compound, acetylating the base, and in many cases chlorinating the resulting anilide in situ. In the case of nitrobenzene, which was our interest, they reported that only p-chloroacetanilide was obtained, and in almost theoretical yield. This was, initially attractive to us, but turned out to be misleading. Numerous experiments gave only 20-30% yield and even modified procedures gave no more than 35% of p-chloroacetanilide. Therefore this short cut method was abandoned.

[2-¹⁴C]-acetanilide was hydrolyzed in acid solution. The radioactivity of the product was measured so as to allow for dilution with p-chloroaniline to give a desired activity of 3.8 mCi/mol. This diluted [2-¹⁴C]-chloroaniline was used for the preparation of [2-¹⁴C]-4,4'-dichloroazobenzene.

Each diluted, labeled 4,4'-dichloroazobenzene was reduced to 4,4'-dichlorohydrazobenzene and used for rearrangement.

Products of Reaction

In acidic 60% aqueous dioxane solution at 0 °C, 4,4'-dichlorohydrazobenzene undergoes the o- and p-semidine rearrangement and concurrent disproportionation to

p-chloroaniline and 4,4'-dichloroazobenzene. These products have been reported earlier,^{7,22} but the quantitative descriptions which were given are quite wrong. For example, 22% of o-semidine, 75% disproportionation and a trace of p-semidine, determined spectroscopically after paper chromatography, were reported.⁷ In the present, work product distributions were determined by HPLC and after separation by flash chromatography. However instead of measuring the distribution of amine products directly, the mixture of amines was converted into a mixture of their trifluoroacetyl derivatives, not only for quantitative analysis but also for isotopic assays.

Conversion of amine products into acetyl or trifluoroacetyl derivatives for isotopic assays offers advantages for both whole-molecule-ion mass spectrometry and scintillation counting. These derivatives are easier to handle for separation and purification and usually give much smaller M-1 peaks, which can interfere with the measurement of the isotope ratio, than do the parent amines themselves. In scintillation counting, the purity of the sample is critical. Therefore utilization of a crystalline derivative allows for purification not only by recrystallization but also by sublimation before counting.

Initially, acetyl derivatives of reaction products were made, but poor separations of the N-acetyl-o-semidine and p-chloroacetanilide were obtained. The R_f values of these

compounds were almost identical in elutions by many solvents or solvent mixtures. A good solvent system could not be found to separate these two compound by column or thick layer chromatography. Therefore, trifluoroacetates were investigated. They could be separated successfully. However trifluoroacetylation gave a different type of derivative for each amine product, as shown in Scheme 13. p-Chloroaniline was converted into p-chlorotrifluoroacetanilide (20), the p-semidine into the bis(trifluoroacetyl) derivative (21), and the o-semidine into the diazole (19). That the secondary position of a diarylamine could be trifluoroacetylated was demonstrated by a model reaction of trifluoroacetic acid with diphenylamine, which gave the trifluoroacetamide derivative.

Scheme 13

Quantitative analyses were carried out by flash chromatography 12 times on the products of the reaction of $[^{15}\text{N}, ^{15}\text{N}']$ -, and $[4,4'\text{-}^{13}\text{C}_2]$ -4,4'-dichlorohydrazobenzene, and by HPLC once on products of reaction of unenriched 14. The initial concentrations of 14 which were used for the rearrangements were in the range of 0.02-0.03 M, and the concentration of perchloric acid was always 0.5 M. To comply with Banthorpe's kinetic conditions, the solutions were also always 0.5 M in lithium perchlorate, and reactions were carried out at 0 °C in 60% aqueous dioxane. Under these conditions, almost all of the 4,4'-dichloroazobenzene which was formed by disproportionation, either at partial or 100% conversion, precipitated from solution (solubility: 6.4 mg in 100 mL of 60% aqueous acidic dioxane at 0 °C). This 4,4'-dichloroazobenzene was filtered and the filtrate was added to a sodium hydroxide solution to quench further reaction. Air was bubbled into the alkaline filtrate to oxidize unreacted 14 to 4,4'-dichloroazobenzene (17). Compound 17 which formed in this manner was filtered. The filtrate, which contained a small amount of 17 together with the products of rearrangement was subjected to a separation procedure. The components were separated by flash chromatography after trifluoroacetylation. The yield of products from reaction of $[^{15}\text{N}, ^{15}\text{N}']$ - and $[4,4'\text{-}^{13}\text{C}]$ -14 are given in Table 3. The yield of 17 is described in two ways. The first column (A) indicates the amount of 17 which was

formed by disproportionation and was filtered from the cold acid solution. The second column (B) indicates the amount of 17 which was formed by air oxidation of unreacted 14. These two yields were used for calculating the extent of a low conversion. In the case of 100% conversion, only one yield is listed and is, solely, that from disproportionation. Amine products were converted into trifluoroacetyl derivatives and analyzed. Their yields are shown in Table 3 as 19, 20 and 21.

As we see from the data in Table 3, the o-semidine is not the only major rearrangement product. The o- and p-semidines are formed in comparable amounts. Another feature is surprising. Two equivalents of p-chloroaniline and one equivalent of 4,4'-dichloroazobenzene (17) should be formed by disproportionation. However, the amount of 17 was always larger than expected. To be certain that this finding, which is based upon isolation and chromatographic separation of the products was correct, direct HPLC analysis of the reaction products was carried out, also. The results are given in the last row in Table 3. The amount of 4,4'-dichloroazobenzene was larger than expected from disproportionation based upon the yield of p-chloroaniline. This suggested that 4,4'-dichloroazobenzene was formed not only from disproportionation but also from another redox reaction.

One possible redox source arises in the formation of the p-semidine. Because a chloronium ion, Cl^+ , or some functional equivalent, should be ejected during p-semidine formation, a redox partner for the Cl^+ is needed. In that case, an amount of 4,4'-dichloroazobenzene equal to that of the p-semidine would be formed and the total amount of 4,4'-dichloroazobenzene would be equatable to the sum of the amounts of p-chloroaniline and p-semidine. The ratio of 4,4'-dichloroazobenzene to the sum of p-chloroaniline and p-semidine in each reaction which proceeded to 100% conversion is given in Table 6 and is seen to be always close to 1.00. This consequence not only affects the material balance in the reaction, but has a role in accounting for the KIE in disproportionation as will be described later. With this correction, the acid-catalyzed reaction of 4,4'-dichlorohydrazobenzene gave approximately 11% of o-semidine, 12% of p-semidine and 60% of disproportionation, accounting for a total of 95% of the starting material (Scheme 14).

KIE Measurements

In order to measure the relative rates of reactions of unlabeled and specifically labeled 4,4'-dichlorohydrazobenzene in a competitive way, a synthetic mixture of 5 mol % enriched 4,4'-dichlorohydrazobenzene (14) in the unenriched

Table 6: The Ratio of the yield of 4,4'-Dichloro-azobenzene to the Sum of the Yields of p-Semidine and p-Chloroaniline.

run	% conversion	$\frac{17}{\frac{20}{+} \frac{21}{}}$		x 100
		<u>20</u>	<u>21</u>	
1	100			97.2
2	100			95.4
3	100			96.2
4	100			98.4
5	100			94.5
6	100			97.9
13	100			104.9

Scheme 14

form was used. The mixture was allowed to react to low and 100% conversions and products were separated and their isotopic compositions were measured in three ways. Scintillation counting was used for ^{14}C labeling, while whole-molecule-ion mass spectrometry (WMIMS) and isotope-ratio mass spectrometry (IRMS) were used for ^{15}N and ^{13}C labeling. The amine products were not used themselves, however, but were converted quantitatively into their mono or bis(trifluoroacetyl) derivatives, which were then separated for measurement of isotopic abundances. The reason for this was initially connected with measuring relative abundances by WMIMS. For this method, the amine products themselves were not suitable because their mass spectra showed substantial (M - 1) peaks, whereas (M - 1) peaks are quite small in the mass spectra of the derivatives. These derivatives were easier to handle for separation and purification by crystallization and sublimation than the original reaction products. Therefore derivatives were also used in the other methods of measuring relative abundance by scintillation counting and IRMS.

Nitrogen KIE were obtained first with the doubly enriched substrate, [^{15}N , $^{15}\text{N}'$]14. For the p-chloroaniline derivative (20) the ratio of the ions (M + 3)/(M + 2), namely 226/225, was measured by high precision, multiscan, whole-molecule-ion mass spectrometry. Although the (M - 1) peak of the 20 was quite small, the effect of the [(M + 2) -

1] abundance was large enough to interfere with measuring the ratio $(M + 1)/M$ because of the large contribution of the $(M + 2)$ abundance due to the ^{37}Cl isotope. To reduce this interference, the ratio of ions $(M + 3)/(M + 2)$ was chosen, because the contribution of $[(M + 4) - 1]$ was much smaller than that of $[(M + 2) - 1]$. Another problem with the analysis of compound 20 was due to its volatility. The Hewlett-Packard mass spectrometer, model 5995, has no cooling system and no regulation system for the solid-sample-inlet, with which the vapor pressure of the sample may be controlled. To maintain a constant pressure, that is, a constant concentration of the compound in the source, a modified solid-sample-inlet probe was used (Figure 1). With this modified probe, the amount of sample introduced into the source could be controlled by a needle valve and probe temperature variation. However, this new probe proved to be difficult to clean. The whole probe became contaminated in a run because the sample was vaporized at one end and was bled into the mass spectrometer from the other end of the probe. This contamination could not be removed by conventional cleaning methods such as washing with solvent or sonicating. Finally, this problem was solved by heating the probe for several hours while it was connected to a vacuum pump.

For the p-semidine derivative 21, the ratio of the $(M + 2)/M$ ions, namely 412/410, was measured with the normal solid-sample-inlet probe and reasonably reproducible results were obtained.

For the o-semidine derivative (19), however, satisfactorily reproducible data could not be obtained by this method because of the difficulty of getting precise $(M + 2)$ enrichment data for molecules containing two chlorine atoms.

The enrichment of mass 226 in 20 and of mass 412 in 21 was then normalized for the natural abundance, because substrate containing only 5 mol % of $[^{15}\text{N}, ^{15}\text{N}']\text{-14}$, had been used. The normalized data were used in the Bigeleisen equation (equation 20)⁷³ for calculating the KIE for two ^{15}N atoms.

$$(k_{\text{H}}/k_{\text{L}} - 1) \ln [1 - f(x/y)] = \ln \left[1 + \frac{R_{\text{AO}} - R_{\text{AF}}}{R_{\text{AO}}} \frac{f(x/y)}{1-f(x/y)} \right] \quad (20)$$

In this equation, H and L designate the heavy and light isotopes respectively, f is the extent of reaction, R_{AO} is the AMU mass ratio at 100% reaction, R_{AF} is the AMU mass ratio at f extent of reaction, x is $(1 + R_{\text{AO}})$, and y is $(1 + R_{\text{AF}})$. The results are given in Tables 7 and 8.

Carbon and nitrogen KIE were also measured by isotope-ratio mass spectrometry. In this method the sample is converted into carbon dioxide and nitrogen gases and the

Table 7: Kinetic Isotope Effects in the Acid-Catalyzed Disproportionation of 4,4'-Dichlorohydrazobenzene.

run	isotope	method	% conv. ^a	KIE _{obs}
1	¹⁵ N, ¹⁵ N'	WMIMS ^b	22.2	1.024 ± 0.0075
2	¹⁵ N, ¹⁵ N'	WMIMS ^b	22.5	1.024 ± 0.0005
3	¹⁵ N, ¹⁵ N'	WMIMS ^b	20.0	1.031 ± 0.0086
5	¹⁵ N ^c	IRMS ^d	24.3	1.0126
6	¹⁵ N ^c	IRMS ^d	14.7	1.0155
5	4,4'- ¹³ C	IRMS ^d	24.3	0.9997
6	4,4'- ¹³ C	IRMS ^d	14.7	1.0039
7	4- ¹⁴ C	SCINT	18.0	1.0000 ± 0.0017
8	4- ¹⁴ C	SCINT	23.0	0.9956 ± 0.0020
9	4- ¹⁴ C	SCINT	17.0	0.9966 ± 0.0024
10	2- ¹⁴ C	SCINT	22.0	1.0019 ± 0.0016
11	2- ¹⁴ C	SCINT	26.0	1.0003 ± 0.0018
12	2- ¹⁴ C	SCINT	18.0	0.9987 ± 0.0027

^a Based on the amount of 4,4'-dichloroazobenzene obtained after air oxidation of unreacted 4,4'-dichlorohydrazobenzene. ^b Whole-molecule-ion mass spectrometry.

^c Naturally-abundant ¹⁵N. ^d Isotope-ratio mass spectrometry. IRMS measurements were single rather than replicate. Therefore, error limits for separate KIE values cannot be given.

Table 8: Kinetic Isotope Effects in the Acid-Catalyzed o-Semidine Rearrangement of 4,4'-dichlorohydrazobenzene.

run	isotope	method	% conv. ^a	KIE _{obs}
5	¹⁵ N ^b	IRMS ^c	24.3	1.0157
6	¹⁵ N ^b	IRMS ^c	14.7	1.0152
5	4,4'- ¹³ C	IRMS ^c	24.3	1.0002
6	4,4'- ¹³ C	IRMS ^c	14.7	0.9984
7	4- ¹⁴ C	SCINT	18.0	1.0050 ± 0.0024
8	4- ¹⁴ C	SCINT	23.0	1.0055 ± 0.0081
9	4- ¹⁴ C	SCINT	17.0	0.9975 ± 0.0031
10	2- ¹⁴ C	SCINT	22.0	0.9978 ± 0.0010
11	2- ¹⁴ C	SCINT	26.0	1.0029 ± 0.0028
12	2- ¹⁴ C	SCINT	18.0	0.9984 ± 0.0032

^a Based on the amount of 4,4'-dichloroazobenzene obtained after air oxidation of unreacted 4,4'-dichlorohydrazobenzene. ^b Naturally-abundant ¹⁵N. ^c Isotope-ratio mass spectrometry. IRMS measurements were single rather than replicate. Therefore, error limits for separate KIE values cannot be given.

mass ratios 45/44 and 29/28 are measured relative to the ratio in arbitrary standards. Ordinarily, this method is best used with a compound whose isotopic content is close to that in the standard. Although a mixture of 5 mol-% of $[4,4'-^{13}\text{C}_2]_{14}$ in 14 was used for reaction, the atom-% enrichment in the samples was much lower (0.63-0.72 atom-% above the normal abundance of ^{13}C) and was suitable to measure the $^{13}\text{C}/^{12}\text{C}$ ratio. The ^{13}C -enriched samples contained no enrichment in ^{15}N . Therefore, they were also suitable for measuring nitrogen mass ratio by IRMS. It was possible, that is, to obtain by isotope ratio mass spectrometry the ratio of masses $^{15}\text{N}/^{14}\text{N}$ as well as $^{13}\text{C}/^{12}\text{C}$ in the ^{13}C enriched products. From these data we were able to calculate the nitrogen KIE for one ^{15}N atom and the carbon KIE for two ^{13}C atoms as follows.

In this method, the mass ratio is obtained as that related to an arbitrary standard and is expressed as a "del" value as shown in equation 21.

$$\delta (^{13}\text{C}_{\text{sample}}) \% = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000 \quad (21)$$

From this equation, the ratio $(^{13}\text{C})/^{12}\text{C})_{\text{sample}}$ was obtained for samples taken at low and 100% conversion, and used for calculating KIE with equation 22.

$$k_L/k_H = \frac{\ln (1 - f)}{\ln (1 - rf)} \quad (22)$$

In equation 22, f is the extent of conversion and r is $(^{13}\text{C}/^{12}\text{C})_{\text{low}}/(^{13}\text{C}/^{12}\text{C})_{\text{high}}$. Analogous treatment was used for $(^{15}\text{N}/^{14}\text{N})_{\text{low}}/(^{15}\text{N}/^{14}\text{N})_{\text{high}}$, the subscripts referring to the percent conversion. Arbitrary standards were normal nitrogen gas with a mass ratio (29/28) of 0.007353, and a carbon source with a mass ratio $^{13}\text{C}/^{12}\text{C}$ of 0.011237. The results thus obtained are given in Tables 7, 8, and 9.

Carbon KIE was also measured for reactions of [4- ^{14}C]- and [2- ^{14}C]-substrates by scintillation counting. KIE were calculated from measurements (cpm/mg) on the low and 100% conversion samples according to equation 23.

$$k_L/k_H = \frac{\ln (1 - f)}{\ln [1 - (fR_p/R_o)]} \quad (23)$$

In equation 23, f is the extent of conversion, R_o is (cpm/mg) in high conversion and R_p is (cpm/mg) in low conversion. The results are given in Tables 7, 8, and 9.

Observed KIE and Real KIE

In some cases the observed KIE which are shown in Tables 7-9 are not the real, intermolecular KIE. This arises because, in the formation of some products, there is an intramolecular competition between labeled and unlabeled

Table 9: Kinetic Isotope Effects in the Acid-Catalyzed p-Semidine Rearrangement of 4,4'-Dichlorohydrazobenzene.

run	isotope	method	% conv. ^a	KIE _{obs}
1 ^e	¹⁵ N, ¹⁵ N'	WMIMS ^b	22.2	1.0187 ± 0.0027
	¹⁵ N, ¹⁵ N'	WMIMS ^b		1.0221 ± 0.0075
2 ^e	¹⁵ N, ¹⁵ N'	WMIMS ^b	22.5	1.0344 ± 0.0025
	¹⁵ N, ¹⁵ N'	WMIMS ^b		1.0310 ± 0.0042
3 ^e	¹⁵ N, ¹⁵ N'	WMIMS ^b	20.0	1.0380 ± 0.0042
	¹⁵ N, ¹⁵ N'	WMIMS ^b		1.0251 ± 0.0037
5	¹⁵ N ^c	IRMS ^d	24.3	1.0157
6	¹⁵ N ^c	IRMS ^d	14.7	1.0166
5	4,4'- ¹³ C	IRMS ^d	24.3	0.9945
6	4,4'- ¹³ C	IRMS ^d	14.7	0.9995
7	4- ¹⁴ C	SCINT	18.0	1.0058 ± 0.0020
8	4- ¹⁴ C	SCINT	23.0	0.9981 ± 0.0020
9	4- ¹⁴ C	SCINT	17.0	1.0004 ± 0.0025

^a Based on the amount of 4,4'-dichloroazobenzene obtained after air oxidation of unreacted 4,4'-dichlorohydrazobenzene. ^b Whole-molecule-ion mass spectrometry.

^c Naturally-abundant ¹⁵N. ^d Isotope-ratio mass spectrometry. IRMS measurements were single rather than replicate. Therefore, error limits for separate KIE values cannot be given. ^e Duplicate assays of mass ratios were made in each run.

positions. This competition occurs in the p-semidine and o-semidine rearrangement of a singly labeled substrate, and affects the carbon KIE. To compensate for this effect the following arithmetical treatment was carried out.

Calculation of carbon KIE in the o-semidine rearrangement. There are four ortho-carbon atoms in 4,4'-dichlorohydrazobenzene which can be involved in o-semidine formation. Among them only one carbon atom is labeled with ^{14}C . Therefore, there is intramolecular competition between three unlabeled and one labeled position in forming the o-semidine (Scheme 15). This must be taken into account in arriving at the derived KIE which signifies intermolecular competition between labeled (k_*) and unlabeled positions (k_1). From this scheme, the observed KIE (KIE_{obs}) is formulated as equation 24.

$$\text{KIE}_{\text{obs}} = (k_1/k_*)_{\text{obs}} = \frac{4k_1}{k_* + k_2 + 2k_3} \quad (24)$$

If we assume (reasonably) that k_2 , k_3 and k_1 have the same magnitude, equation 24 can be simplified to equation 25 and the real KIE (k_1/k_*) can be calculated as shown in equation 26. Results are given in Table 10 where they are expressed

$$\text{KIE}_{\text{obs}} = \frac{4k_1}{k_* + 3k_1} \quad (25)$$

Scheme 15

$$\text{KIE}_{\text{real}} = k_1/k_* = \frac{\text{KIE}_{\text{obs}}}{4 - 3\text{KIE}_{\text{obs}}} \quad (26)$$

in comparison with KIE_{obs} . The real KIE values are about four times larger than the observed KIE.

Table 10: Observed and Calculated Kinetic Isotope Effects ($k^{12}\text{C}/k^{14}\text{C}$) in the o-Semidine Rearrangement.

run	isotope	method	$\text{KIE}_{\text{obs}}^{\text{a}}$	KIE_{real}
10	2- ^{14}C	SCINT	0.9978 ± 0.0010	0.9913 ± 0.0039
11	2- ^{14}C	SCINT	1.0029 ± 0.0028	1.0117 ± 0.0114
12	2- ^{14}C	SCINT	0.9984 ± 0.0032	0.9936 ± 0.0126

^a Observed KIE. ^b Calculated KIE based on equation 26.

Calculation of carbon KIE in the p-semidine rearrangement. For the p-semidine rearrangement with singly labeled substrate, an intramolecular competition reaction must also be considered. There are two possible pathways for rearrangement in the singly labeled molecule, as shown in Scheme 16. The observed KIE (KIE_{obs}) is shown in equation 27 and can be simplified as equation 28, based on the assumption that k_1 and k_2 are the same.

$$\text{KIE}_{\text{obs}} = (k_1/k_*)_{\text{obs}} = \frac{2k_1}{k_* + k_2} \quad (27)$$

$$\text{KIE}_{\text{real}} = k_1/k_* = \frac{\text{KIE}_{\text{obs}}}{2 - \text{KIE}_{\text{obs}}} \quad (28)$$

The real KIE are calculated according to equation 28 and the results are given in Table 11.

When substrate which was doubly labeled in the p-position with ^{13}C was used, there was no problem with intramolecular competition. However, when the isotope ratios were measured by IRMS another treatment was needed and will be discussed later.

Scheme 16

Table 11: Observed and Calculated Kinetic Isotope Effects ($k^{12}\text{C}/k^{14}\text{C}$) in the p-Semidine Rearrangement.

run	isotope	method	KIE _{obs} ^a	KIE _{real} ^b
7	4- ¹⁴ C	SCINT	1.0058 ± 0.0020	1.0117 ± 0.0040
8	4- ¹⁴ C	SCINT	0.9981 ± 0.0020	0.9962 ± 0.0040
9	4- ¹⁴ C	SCINT	1.0004 ± 0.0025	1.0008 ± 0.0050

^a Observed KIE. ^b Calculated KIE based on equation 28.

Calculation of carbon KIE in the disproportionation.

The carbon KIE for the formation of p-chloroaniline can be calculated in two ways. When [2-¹⁴C]-4,4'-dichlorohydrazobenzene was used, the carbon KIE could be seen only if disproportionation occurred via the same intermediate which is involved in o-semidine formation. Therefore the real KIE for disproportionation is calculated in the same way (equation 26) as was used utilized for calculating KIE_{real} for o-semidine formation. When [4-¹⁴C]-4,4'-dichlorohydrazobenzene was used, carbon KIE could be realized if either a p-semidine intermediate or a benzidine-type intermediate were involved. If a benzidine-type intermediate is involved, no further treatment is needed because both para carbon atoms are involved. But if the p-semidine intermediate is involved, real KIE must be calculated in the same way (equation 28) as in the

calculation of KIE_{real} for p-semidine formation. The results are given in Table 12.

Table 12: Observed and Calculated Kinetic Isotope Effects ($k^{12}C/k^{14}C$) in Disproportionation.

run	isotope	method	KIE_{obs}^a	KIE_{real}
7	4- ^{14}C	SCINT	1.0000 ± 0.0017	1.0000 ± 0.0034^b
8	4- ^{14}C	SCINT	0.9956 ± 0.0020	0.9912 ± 0.0040^b
9	4- ^{14}C	SCINT	0.9966 ± 0.0024	0.9932 ± 0.0048^b
10	2- ^{14}C	SCINT	1.0019 ± 0.0016	1.0076 ± 0.0065^c
11	2- ^{14}C	SCINT	1.0003 ± 0.0018	1.0012 ± 0.0072^c
12	2- ^{14}C	SCINT	0.9987 ± 0.0027	0.9948 ± 0.0107^c

^a Observed KIE. ^b Calculated KIE based on equation 28.

^c Calculated KIE based on equation 26.

Calculation of carbon KIE for disproportionation and p-semidine formation in reactions of $[4,4'-^{13}C_2]_{-14}$. IRMS gives only the average $^{13}C/^{12}C$ ratio of all carbon atoms in the molecule. We are interested only in the changes in isotope ratio which may occur at the labeled carbon atom. Therefore, to isolate the labeled carbon atom arithmetically the following treatment was carried out. The total isotope content can be formulated as equation 29. In this equation,

$$n(^{13}C/^{12}C)_{sample} = (n - x)(^{13}C/^{12}C)_{nat} + x(^{13}C/^{12}C)_* \quad (29)$$

n is total number of carbon atoms in compound, x is number of labeled carbon atoms, $(^{13}\text{C}/^{12}\text{C})_{\text{sample}}$ is the average isotope content of the sample which is measured by IRMS and is calculated from the measured "del" values (equation 21), $(^{13}\text{C}/^{12}\text{C})_{\text{nat}}$ is the average isotope content of the unlabeled sample, and $(^{13}\text{C}/^{12}\text{C})_*$ is the isotope content of the labeled carbon atoms of the labeled sample. From this equation $(^{13}\text{C}/^{12}\text{C})_*$ can be calculated for low and high conversions and used in equation 22 for KIE calculation. The results are shown in Table 13.

Table 13. Recalculated Carbon Kinetic Isotope Effects ($k^{12}\text{C}/k^{13}\text{C}$) in the p-Semidine Rearrangement and Disproportionation.

run	isotope	method	type of reaction	KIE _{obs} ^a	KIE _{real} ^b
5	4,4'- ¹³ C	IRMS	p-semidine rearr.	0.9945	0.9878
6	4,4'- ¹³ C	IRMS	p-semidine rearr.	0.9995	0.9986
5	4,4'- ¹³ C	IRMS	disproportionation	0.9997	0.9992
6	4,4'- ¹³ C	IRMS	disproportionation	1.0039	1.0088

^a Observed KIE. ^b Recalculated KIE based on equation 29 and equation 22.

The Meaning of the KIE

Nitrogen KIE

Each of the reactions, disproportionation and rearrangement to o- and p-semidine, showed substantial nitrogen KIE (Table 14). The size of the KIE of double labeling is close to the square of the KIE for single labeling and this gives us confidence about the validity of the KIE values. The nitrogen KIE for these three reactions are hardly distinguishable from each other. This implies that there is no substantial difference between the transition states of these reactions. In the o-semidine rearrangement, a concerted 1,3-sigmatropic shift is forbidden according to the rules of conservation of orbital symmetry. This rearrangement must be dissociative if it complies with these rules. The substantial observed nitrogen KIE reflects a N-N bond cleavage in the rate-determining step, and the absence of a carbon KIE supports the dissociative process, which will be discussed further later. In the p-semidine rearrangement, a 1,5-sigmatropic shift is allowed and therefore this rearrangement can be a concerted process. If the reaction is concerted, then the nitrogen KIE for p-semidine formation reflects the sum of N-N bond cleavage and C-N-C bond formation. However, the observed nitrogen KIE for the o- and p-semidine formation (1.0155 and 1.0162) are almost the same and this suggests that the transition states

are the same; that is, both dissociative. The carbon KIE also support this conclusion.

Table 14: Summary of Averaged Nitrogen kinetic Isotope Effects ($k^{14}\text{N}/k^{15}\text{N}$) in Acid-Catalyzed Reactions of 4,4'-Dichlorohydrazobenzene.

reaction	KIE and basis		
	$^{15}\text{N}, ^{15}\text{N}^{\text{a}}$	$^{15}\text{N}^{\text{b}}$	$(^{15}\text{N})^2$
disproportn.	1.0260 ± 0.0028	1.0141 ± 0.0015	1.0284
o-semidine rearr.		1.0155 ± 0.0003	
p-semidine rearr.	1.0282 ± 0.0068	1.0162 ± 0.0005	1.0327

^a WMIMS ^b IRMS

Carbon KIE

No carbon KIE is found other than unity, effectively, in all three reactions (Table 15). Each reaction will be discussed separately as follows.

The o-semidine rearrangement. In principle, there should not be a carbon KIE for this 1,3-sigmatropic shift. In the present work, the carbon KIE was measured using [2- ^{14}C]14 and found to be 0.9989, which is considered to be unity. This is supported, insofar as confidence in the experimental procedure is concerned, by the observed ^{14}C KIE for o-semidine formation from [4- ^{14}C]14. That, of course,

Table 15: Summary of Averaged Carbon Kinetic Isotope Effects in Acid-Catalyzed Reactions of 4,4'-Dichlorohydrazobenzene.

reaction	KIE and basis		
	$2\text{-}^{14}\text{C}$	$4\text{-}^{14}\text{C}$	$4,4'\text{-}^{13}\text{C}^a$
dispro- portn.	1.0012 ± 0.0082	0.9948 ± 0.0040	1.0040 ± 0.0048
o-semidine	0.9989 ± 0.0093	1.0027 ± 0.0042	0.9993 ± 0.0009
p-semidine		1.0029 ± 0.0043	0.9934 ± 0.0056

^a IRMS.

cannot be anything but unity, and the average result (1.0027) gives a measure of precision. However, as discussed earlier, the observed KIE is about one fourth of the real KIE with singly labeled 14, and a small, real KIE might not be seen when expressed as KIE_{obs} . Ideally, $[2,2',6,6'\text{-}^{14}\text{C}_4]\text{14}$ would be the best substrate for the KIE measurement in o-semidine formation, where KIE_{obs} would be the same as KIE_{real} . Practically, however, it is impossible to make such a substrate because isotopically pure $[2,6\text{-}^{14}\text{C}]\text{-aniline}$ would be needed for the preparation of $[2,2',6,6'\text{-}^{14}\text{C}_4]\text{14}$. In order to obtain a more confident result, doubly labeled 14 may be used. A ^{13}C KIE of 0.9993 was measured using $[4,4'\text{-}^{13}\text{C}_2]\text{14}$, that is, 14 which had ^{13}C enrichments in its two p-positions. Since these positions

are not involved in o-semidine formation the ^{13}C KIE for the o-semidine derives only from naturally abundant ^{13}C in the o-positions, and is also considered to be unity. This carbon KIE together with the nitrogen KIE suggests that the o-semidine is formed in a dissociative way, that is, that an intermediate is formed in the rate-determining step. A possible candidate may be the π -complex, or a caged-radical intermediate.

The p-semidine rearrangement. The ^{14}C KIE (1.0029) is considered to be unity, and ^{13}C KIE (0.9934) measured with $[4,4'-^{13}\text{C}_2]\underline{14}$ is considered to be either unity or very small and inverse. The p-semidine rearrangement is a 1,5-sigmatropic shift. In principle, this can be a concerted process which would exhibit a carbon KIE. Earlier, it was found that the p-semidine rearrangement of 4-methoxyhydrazobenzene (4) is a concerted one, exhibiting both substantial nitrogen and carbon KIE.³ Although the two p-semidine rearrangements are the same in form, they differ considerably in character. Rearrangement of 4 is first order in acid, and a proton is displaced from its p-position. Rearrangement of 14 is second order in acid and Cl^+ is displaced. It is not certain that these differences lead to a difference in transition states in the p-semidine rearrangements of 4 and 14. The carbon KIE together with nitrogen KIE suggests that the p-semidine rearrangement of 14 is not a concerted process. We are not really able to

make a conclusion with certainty for the p-semidine rearrangement because in a bond-forming reaction, KIE may be smaller than, greater than, or equal to unity. An intermediate 45 is certainly formed, but the pathway to it is not clear. p-Semidine formation requires the loss of a chlorine atom from the p-position of 14 to which the new C-N bond has been made. The chlorine atom must leave effectively as Cl^+ , and the proposal for the way in which this happens is linked to the disproportionation reaction shown in Scheme 17.

Disproportionation. Disproportionation reactions have the same kinetic characteristics as the rearrangements they accompany. In this case, disproportionation is first order in 14 and second order in acid. The kinetic character and stoichiometry of the disproportionation reaction require that an intermediate is formed from one molecule of 14 in the rate-determining step, and next undergoes a rapid redox reaction with a second molecule of 14. Among several possible intermediates, a 4,4'-quinonoidal one (e.g., 46 in Scheme 18) has been signified in earlier work by the finding of substantial carbon and nitrogen KIE in the disproportionation of 4,4'-diiodohydrazobenzene.⁵³ A similar result was expected from disproportionation of 14. However the finding of a substantial nitrogen KIE but no carbon KIE from using $[4,4'\text{-}^{13}\text{C}_2]\text{14}$ indicates that a 4,4'-quinonoidal intermediate is not involved in the

disproportionation of 14. The carbon result suggests that, probably, the intermediate in this case is instead the quinonoid of either the p- or o-semidine (Schemes 17 and 19), since these are formed without exhibiting carbon KIE. The relative amounts of 4,4'-dichloroazobenzene and 4-chloroaniline which were obtained from 14 have a bearing on these possibilities. An excess of 4,4'-dichloroazobenzene was obtained over that of 4-chloroaniline. This excess may have come only from the reaction between the quinonoid intermediate of p-semidine formation and 14. From this we can deduce that the proximity of this quinonoid intermediate and 14 would also lead to disproportionation. Once this intermediate 45 is formed in the rate-determining step, its reaction with a second molecule of 14 can occur in two ways: by C-C scission for disproportionation and by Cl^+ abstraction for p-semidine formation. The latter route provides the azo compound 17 in excess of that from the disproportionation reaction, as observed (Table 3).

Scheme 17

45

Scheme 18

Scheme 19

CHAPTER IV

SUMMARY AND CONCLUSIONS

In acidic 60% aqueous dioxane solution at 0 °C, 4,4'-dichlorohydrazobenzene (14) underwent concurrent disproportionation to form p-chloroaniline and 4,4'-dichloroazobenzene and o- and p-semidine rearrangement. In the p-semidine rearrangement one of the chlorine atoms of 14 is displaced, in essence, as Cl^+ . This requires participation of a second molecule of 14 in a redox reaction. The overall fate of 14, therefore, was to give 11% of o- and 12% of p-semidine rearrangement (along with 12% of 4,4'-dichloroazobenzene), and 60% of disproportionation, which accounts for 95% of 14.

Nitrogen and carbon kinetic isotope effects (KIE) were determined for each of these reactions, with the use of [$^{15}\text{N}, ^{15}\text{N}'$]14, [$2\text{-}^{14}\text{C}$]14, [$4\text{-}^{14}\text{C}$]14, and [$4,4'\text{-}^{13}\text{C}_2$]14. Isotope ratios were measured in the trifluoroacetyl derivatives of the o- and p-semidine and p-chloroaniline, with a combination of scintillation counting, whole-molecule-ion mass spectrometry (WMIMS), and isotope-ratio mass spectrometry (IRMS). Nitrogen KIE were obtained by WMIMS for two ^{15}N atoms in disproportionation (1.0260) and p-semidine rearrangement (1.0282) and by IRMS for one (naturally abundant) ^{15}N atom in disproportionation (1.0141)

and o- (1.0155) and p-semidine (1.0162) rearrangement. The ^{13}C (IRMS) and ^{14}C KIE were measured for all reactions, but in no case was a KIE other than unity obtained. These results show that o-semidine formation from 14 complies with the expectation that a [1,3]-sigmatropic shift should not be a concerted process. Although a concerted [1,5]-sigmatropic shift is possible, the p-semidine results suggest that the p-semidine rearrangement of 14 does not proceed by a concerted process. However, in this case, the relevant KIE are for a bond-forming reaction, so that a firm decision either for or against concertedness is not possible. Finally, the results indicate that disproportionation involves one (or both) of the two semidine rearrangement intermediates (45, 47). The most likely one is that of the p-semidine rearrangement (45). A rapid redox reaction of the quinonoid intermediate of the p-semidine rearrangement, formed in the rate-determining step, with a second molecule of 14 can then lead to the p-semidine by removal of Cl^+ , and to disproportionation by scission of the central C-C bond of the intermediate. These paths account for the distribution of the products formed and the KIE of their formation.

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