

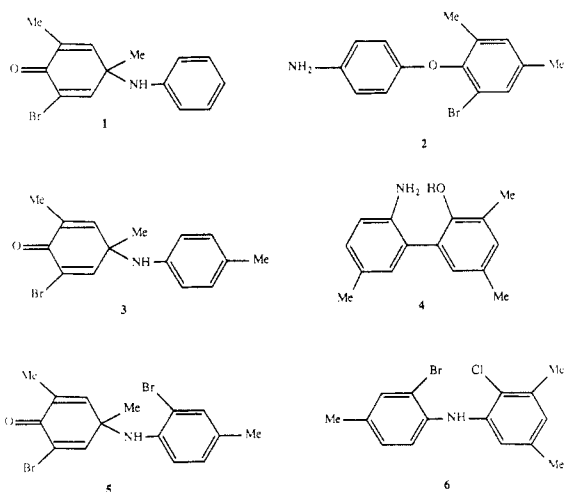
The Mechanism of the Quinamine Rearrangements

Bogdan Boduszek¹ and Henry J. Shine*

Contribution from the Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409. Received October 13, 1987

Abstract: Acid-catalyzed rearrangement of 6-bromo-2,4-dimethyl-4-(phenylamino)cyclohexa-1,4-dienone (**1**, a quinamine) in aqueous methanol gives, from a so-called quinamine rearrangement, 4'-amino-6-bromo-2,4-dimethyldiphenyl ether (**2**) and a number of byproducts. The ratio of yield of **2** to that of byproducts is 76:24. The byproducts are, mostly, 1,3-dimethylcarbazole (**7**) and some of its derivatives, the relative yields of which depend on the concentration of the catalyzing acid, HCl. The major byproduct in low HCl concentrations is 1,3-dimethyl-4-methoxycarbazole (**9**). Kinetic isotope effects (KIE) were measured for the formation of **2** from **1**, which was labeled at the carbonyl oxygen atom ($[^{18}\text{O}]\text{-1}$), the nitrogen atom ($[^{15}\text{N}]\text{-1}$), and the para position of the aniline ring ($[4\text{-}^{14}\text{C}]\text{-1}$). The KIE (averages) were as follows: $k(^{16}\text{O})/k(^{18}\text{O})$, 1.0399; $k(^{14}\text{N})/k(^{15}\text{N})$, 1.0089; $k(^{12}\text{C})/k(^{14}\text{C})$, 1.0501. The results suggest that the formation of **2** is a concerted process, a [5,5]-sigmatropic rearrangement, and not a two-step one, going through the rate-determining formation of a π -complex. KIE were measured for the formation of both **2** and **9** from **1**, which was labeled in the ortho position of the anilino ring ($[2\text{-}^{14}\text{C}]\text{-1}$). The KIE [$k(^{12}\text{C})/k(^{14}\text{C})$] were respectively 0.9895 and 1.0697. These results suggest that the byproduct (**9**) is formed by a concerted process, too, a [3,3]-sigmatropic rearrangement to an intermediate (**14**), which continues on to **9** and the other byproducts. The results show also that **2** cannot be formed from **1** by a succession of two [3,3]-sigmatropic rearrangements, the first of which is to **14**. Thus, the quinamine rearrangements, on the basis of our results with **1**, appear to be concerted, rather than π -complex intermediate, processes.

Quinamine rearrangements are the acid-catalyzed conversions of (arylamino)cyclohexadienones. They are part of the rich collection of rearrangements of N-substituted arylamino compounds, which were brought to light in the early 1900s and which are classified as aromatic rearrangements.² Most of what is known about the scope of the quinamine rearrangements is to be found in the early publications of Fries and co-workers.³⁻⁵ All of the quinamines whose rearrangements have been reported carry a number of substituents in their rings, and the substituents affect which of the two major pathways rearrangement may take. Thus, the quinamine **1** rearranges into the 4-aminodiphenyl ether **2**.³ When a quinamine has a substituent, e.g., methyl, in the para position of the arylamino ring, rearrangement may take a second pathway, leading to a biphenyl, with the expulsion of a labile group from the cyclohexadienone ring. For example, the quinamine **3** rearranged into the biphenyl **4** in 80% yield.⁵ There is also a third, less common pathway available to some acid-catalyzed quinamine rearrangements, that is, the formation of a diphenylamine. An example given by Fries is the rearrangement of **5** into **6**, also in 80% yield.⁵



Apparently, after Fries' work no further interest was shown in the quinamine rearrangements for 30 years, when their study was taken up by Miller. In 1964, Miller showed that rearrangements of quinamines into diphenyl ethers, that is, such as **1** into **2**, are intramolecular and first order in quinamine and acid.⁶

Miller noted that rearrangements into diphenyl ethers had a resemblance to the Claisen rearrangements and could therefore, like them in principle, occur in two successive, intramolecular steps. However, the stepwise mechanism of rearrangement was discounted in favor of one involving a π -complex. In support of the proposal for a π -complex intermediate, Miller noted the strong analogy between the quinamine and benzidine rearrangements, for which a π -complex mechanism has been advocated earlier by Dewar.⁷ Arguments were presented, therefore, that the rearrangement of a quinamine might also occur via a π -complex intermediate.⁸ Somewhat later, Miller pointed out that rearrangement to a diphenyl ether could, in fact, occur by a single, concerted process.⁹ In modern terminology this proposal would be equated to a [5,5]-sigmatropic shift. While acknowledging that all three rearrangements (to diphenyl ether, biphenyl and diphenylamine) could proceed by different pathways, Miller returned to the π -complex intermediate, in that it was attractive to assume that all three types of quinamine rearrangement could proceed through different orientations of the halves of a single π -complex intermediate. Again, the analogy between the quinamine and benzidine rearrangements was made.⁹

Research into the mechanism of the benzidine rearrangement has been replete with controversy. In particular, the π -complex mechanism has received a great deal of criticism.^{2,10-12} In discussing the mechanism of the quinamine rearrangements, one of us noted long ago that whether or not a π -complex was formed in that or the benzidine rearrangement was, then, a matter of opinion.¹³ Recently, prompted by the concepts of pericyclic rearrangements, which were not fully known in the earlier era,

(6) Miller, B. J. *Am. Chem. Soc.* **1964**, *86*, 1127.(7) Dewar, M. J. S. In *Molecular Rearrangements*; de Mayo, P., Ed.; Interscience: New York, 1963; Vol. 1, p 323 and earlier publications.(8) Miller, B. J. *Am. Chem. Soc.* **1964**, *86*, 1135.(9) Miller, B. In *Mechanisms of Molecular Migrations*; Thyagarajan, B. S., Ed.; Interscience: New York, 1968; p 306.(10) Shine, H. J. In *MTP International Review of Science. Aromatic Compounds*; Organic Chemistry, Series 1; Zollinger, H., Ed.; Butterworths: London, 1973; Vol. 3, pp 65-103.(11) Shine, H. J. In *MTP International Review of Science. Aromatic Compounds*; Organic Chemistry, Series 2; Zollinger, H., Ed.; Butterworths: London, 1976; Vol. 3, pp 87-124.(12) Cox, R. A.; Buncel, E. In *The Chemistry of the Hydrazo Azo and Azoxy Groups*; Patai, S., Ed.; Wiley: New York, 1975; pp 775-859.

(13) Reference 2, p 182.

(1) On leave from the Institute for Organic and Physical Chemistry, Technical University, Wroclaw, Poland.

(2) Shine, H. J. *Aromatic Rearrangements*; Elsevier: New York, 1967.(3) Fries, K.; Oehmke, G. *Justus Liebigs Ann. Chem.* **1928**, *462*, 1.(4) Fries, K.; Kuester, A. *Justus Liebigs Ann. Chem.* **1929**, *470*, 20.(5) Fries, K.; Böker, R.; Wallbaum, F. *Justus Liebigs Ann. Chem.* **1934**, *509*, 73.

we used heavy-atom kinetic isotope effects (KIE) to show that the benzidine rearrangement is, in fact, a [5,5]-sigmatropic shift and does not go through a π -complex intermediate.¹⁴ The same technique was used, also, to show that other members of the family of benzidine rearrangements (formation of diphenyls, *o*-benzidines, *o*- and *p*-semidines) were governed by the principles of sigmatropic rearrangements.¹⁵⁻¹⁹ Thus, the erstwhile attractive proposal that all types of benzidine rearrangement could proceed from π -complex intermediates was shown to be invalid. Consequently, we felt that it was questionable that a π -complex mechanism would govern the quinamine rearrangements and that it was appropriate to begin applying the measurement of heavy-atom KIE to studies of these rearrangements, too.

Almost all of the quinamines whose rearrangements have been reported carry one or more bromine atom substituents. Others carry chlorine atoms, instead. Because we use a mass-spectrometric method in measuring the KIE of stable isotopes, it was necessary to avoid the potential complexity of having two or more halogen substituents in the quinamine rings. Furthermore, it was desirable to use a quinamine whose kinetics of rearrangement had been reported, so that we would have a guide to the times of terminating conversions into product. Therefore, we chose for study the rearrangement of **1** into **2**. We now report that our results are not consistent with a mechanism that calls for the rate-determining formation and subsequent rapid collapse of a π -complex but instead with one in which a [5,5]-sigmatropic shift occurs. We are also able to report on the other products, besides **2**, that are formed in the reaction of **1** with acid and on the KIE and mechanism of formation of one of these other products, namely **9**.

Results and Discussion

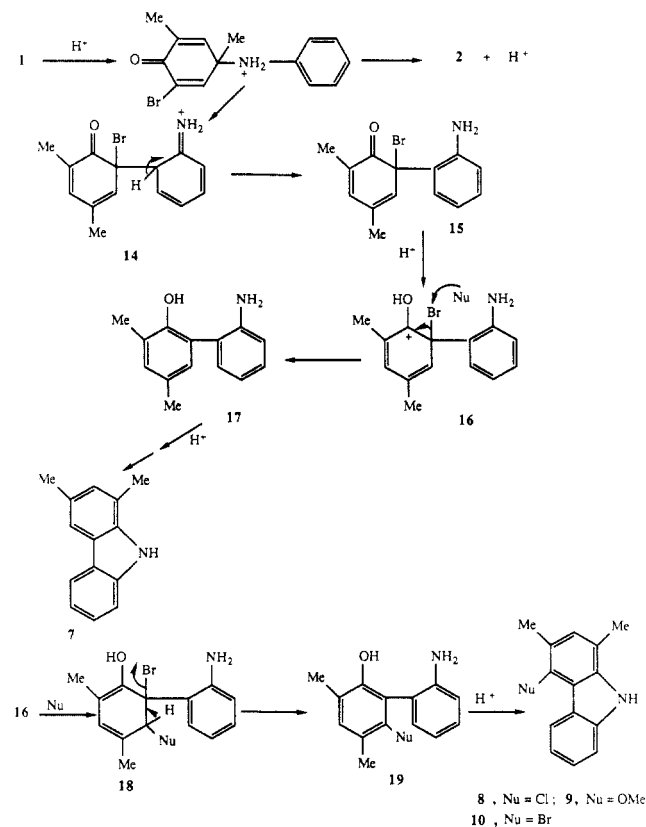
Products of Rearrangement. When carried out as described by Fries and Oehmke,³ in an acetic acid solution of hydrochloric acid, rearrangement of **1** gave only the diphenyl ether product (**2**) in 65% yield. Rearrangement in a much more dilute aqueous methanol solution of hydrochloric acid, in conditions similar to those described by Miller,⁶ gave not only **2** but also a mixture of five byproducts. Fortunately, it was possible to separate **2** from the mixture by column chromatography. It was not possible to separate all of the five byproducts from each other.

Byproducts from other quinamine rearrangements, but not of **1**, have been reported by earlier workers. We are not certain of the structures of all of the byproducts from **1**, but by analogy with earlier work, and on the basis of GC/MS and NMR data, four of the byproducts appear to be 1,3-dimethylcarbazole (**7**) and its monochloro (**8**), monomethoxy (**9**), and monobromo (**10**) derivatives, while the fifth byproduct, on the basis of GC/MS data only, appears to be a dibromo derivative (**11**) of 2'-amino-3,5-dimethyl-2-hydroxybiphenyl (**17**).

The major byproducts were **8** and **9**. Their relative yields depended, however, on the concentration of hydrochloric acid that was used for rearrangement. Estimates of the yields of the byproducts were made from the GC/MS data of mixtures of byproducts obtained from rearrangement of **1** in 2×10^{-1} M hydrochloric acid, and were as follows: **7** (1.1%), **8** (7.7%), **9** (6.5%), **10** (0.14%), and **11** (0.28%). Thus, together with **2** (75%) the products accounted for 89.7% of **1** in this case.

When rearrangement was carried out in 6×10^{-3} M hydrochloric acid, little of **8** was obtained and the major byproduct was **9**. From such rearrangements enough of **9** was isolated for purification, mp 134.5–135.5 °C, and characterization by ¹H and

Scheme I



¹³C NMR spectroscopy. The data are consistent with the structure 1,3-dimethyl-4-methoxycarbazole. Enough of **8** was also isolated for purification, mp 94–96 °C, and characterization by ¹H NMR spectroscopy as 1,3-dimethyl-4-chlorocarbazole. By analogy, **10** is thought to be the 4-bromo derivative, too.

It is possible to rationalize the formation of the major product (**2**) and most of the byproducts as shown in Scheme I. This scheme shows two sigmatropic rearrangements, a [5,5] leading to **2** and a [3,3] leading to the intermediate **14**, from which, subsequently, the several carbazoles (**7**–**10**) are derived. Evidence for the sigmatropic rearrangements is presented later. The scheme helps us to understand why the relative yields of **8** and **9** depended on the concentration of hydrochloric acid (i.e., chloride ion) used for rearrangement, with more of **8** being formed the greater the concentration of chloride ion. Product **17**, which corresponds with some of the biphenyl-type products reported in other rearrangements by Fries,^{3,5} was itself not isolated from our rearrangements, but small amounts were detected by TLC at slightly higher *R_f* than **2**.

Our product analyses show that **2** is by no means the only product obtained from rearrangement of **1**. To our knowledge the products of no other quinamine rearrangement have been documented to this extent. We have established the relative yields of **2** and other products in rearrangements that were carried out for making KIE measurements on **2** alone. That is, the ratio of **2** to all byproducts was measured 14 times and averaged 76:24. We discuss this part of our work below. Our measurements of KIE are of two kinds. KIE for the formation of **2** only were measured with [¹⁸O]-**1**, [¹⁵N]-**1**, and [4-¹⁴C]-**1**. The sites of labeling in these cases were, respectively, the carbonyl oxygen atom, the amino nitrogen atom, and the 4-position of the anilino ring. In these cases no other product was isolated for KIE measurement. KIE were also measured for formation of both **2** and **9** when [2-¹⁴C]-**1** was used, the labeling here being in the 2-position of the anilino ring. It is evident that in this case the carbon atom that became bonded in **2** was unlabeled.

¹⁸O, ¹⁵N, and 4-¹⁴C KIE. Because only **2** was isolated for KIE measurements, it was necessary to separate **2** from all other products and, in low conversion cases, from unreacted **1**, too.

(14) Shine, H. J.; Zmuda, H.; Park, K. H.; Kwart, H.; Horgan, A. G.; Brechbiel, M. *J. Am. Chem. Soc.* **1982**, *104*, 2501.

(15) Shine, H. J.; Zmuda, H.; Kwart, H.; Horgan, A. G.; Brechbiel, M. *J. Am. Chem. Soc.* **1982**, *104*, 5181.

(16) Shine, H. J.; Zmuda, H.; Kwart, H.; Horgan, S. G.; Brechbiel, M. *J. Am. Chem. Soc.* **1982**, *104*, 5181.

(17) Shine, H. J.; Gruszecka, E.; Subotkowski, W.; Brownawell, M.; San Filippo, J., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 3218.

(18) Shine, H. J.; Kupczyk-Subotkowska, L.; Subotkowski, W. *J. Am. Chem. Soc.* **1985**, *107*, 6674.

(19) Rhee, E. S.; Shine, H. J. *J. Am. Chem. Soc.* **1986**, *108*, 1000.

Table I. Kinetic Isotope Effects for the Acid-Catalyzed Rearrangement of **1** into **2**

isotope	conversion, <i>F</i>		KIE (<i>k_L</i> / <i>k_H</i>)	
	A ^a	B ^b	C ^c	D ^d
¹⁵ N	0.063	0.061	1.0099 ± 0.0060	
¹⁵ N	0.13	0.116	1.0081 ± 0.0013	
¹⁵ N	0.38	0.41	1.0087 ± 0.0030	
¹⁸ O	0.10	0.106	1.0432 ± 0.0020	1.0426
¹⁸ O	0.15	0.18	1.0396 ± 0.0023	1.0387
¹⁸ O	0.20	0.20	1.0393 ± 0.0057	1.0383
¹⁸ O	0.25	0.25	1.0374 ± 0.0048	1.0362
4- ¹⁴ C	0.10	0.053	1.0520 ± 0.0017	1.0513
4- ¹⁴ C	0.10	0.075	1.0709 ± 0.0039	1.0700
4- ¹⁴ C	0.20	0.145	1.0425 ± 0.0027	1.0413
4- ¹⁴ C	0.30	0.33	1.0351 ± 0.0025	1.0337

^a Based on kinetic data. ^b Based on the amount of **2** that was isolated. The percent yield of **2** was multiplied by 1.32 to give the total conversion of **1** into **2** and byproducts. The factor 1.32 was obtained from the average of ratios (76:24) of **2** to byproducts obtained from 100% conversions of **1**; see text. ^c Based on kinetic conversions, column A. ^d Based on the conversions *F₁*; see text.

Fortunately, this was easily achieved in one-pass column chromatography. In all, 14 experiments were carried out, comprising three controls with unlabeled **1**, four each with [¹⁸O]-**1** and [4-¹⁴C]-**1**, and three with [¹⁵N]-**1**. Each of these experiments consisted of a low and 100% conversion. Separation of **2** from byproducts as a group was clean in the 100% conversions. From 14 runs, the ratio 2:byproducts averaged 76:24. In the low conversions, **2** was separated without difficulty from a mixture of unrearranged **1** and byproducts. Because **2** was not the only product, its isolation in low conversions did not represent the real extent of conversion of **1**. In order to calculate the real conversion based on isolated products and compare it with the conversion based on rate constant, we assumed that the ratio 2:byproducts would be the same in low as in 100% conversions. Therefore, the extent of a low conversion was obtained by multiplying the conversion into **2** alone by the factor 1.32. The comparison of kinetic and product-based conversions is given in Table I, where it is seen that they are for the most part in reasonable agreement.

The KIE listed in Table I are consistent with the formation of **2** from protonated **1** by a [5,5]-sigmatropic shift. The KIE are associated with breaking the bond to the nitrogen atom and forming a bond between the carbonyl oxygen atom and carbon atom of the para position. The KIE are not consistent with the rate-determining formation of a π -complex, which, later, collapses rapidly after adopting an appropriate configuration for product formation. Thus, this distinction between mechanisms is like that which has been made for the benzidine rearrangement.¹⁴ The relative sizes of the KIE, that is, the small nitrogen and large oxygen and carbon KIE, indicate, in this view, that the extents of bond breaking and bond forming in the transition state are unequal. Only a more sophisticated treatment of the transition-state geometry would give further insight into the relative sizes of the KIE listed in the table. Another explanation of the KIE, arising from their relative sizes should also be considered, that is, that a π -complex is formed in equilibrium with protonated **1** and is converted into **2** in a second, rate-determining step. In this view, the low nitrogen KIE would, in fact, be an equilibrium isotope effect (EIE), while the oxygen and carbon isotope effects would be kinetic. The EIE, here, would, in essence, be for the attack of aniline dication on the sp² carbon atom of the para position of a phenoxide ring. We know of no EIE data analogous to this situation on which we can call for guidance. On the other hand, Yamataka and co-workers have calculated the EIE for addition of ammonia and methylamine to an sp² carbonyl carbon atom, and these EIE are inverse to the extent of ca. 3%.²⁰ EIE for methyl transfer to pyridine and some of its derivatives, in their reactions with various CH₃X, have been estimated to be inverse on the basis of the inverse EIE for proton transfer to the pyri-

Table II. Kinetic Isotope Effects for the Acid-Catalyzed Rearrangement of [2-¹⁴C]-**1** into **2** and **9**

run	conversion, <i>F</i> ^a	KIE [<i>k</i> (¹² C)/ <i>k</i> (¹⁴ C)]	
		2	9 ^b
1	0.114	0.9972 ± 0.0029	
2	0.3	0.9806 ± 0.0034	1.0498 ± 0.0120
3	0.3		1.0614 ± 0.0055
4	0.3	0.9908 ± 0.0041	1.0979 ± 0.0057

^a Based on kinetic data. ^b Adjusted for intramolecular competition.

dines.^{21,22} The EIE for proton transfer to aniline should also be inverse. For example, Tanaka and co-workers²³ have reported the ratio ¹⁴K_a/¹⁵K_a (the EIE for dissociation of the anilinium ions) to be 1.019, meaning in the context of proton transfer to aniline an EIE of 0.981. If we assume that the EIE for π -complex formation may also be represented in the limit by the protonation EIE, we again arrive at an inverse result. Consequently, although we cannot rule out the equilibrium route to **2** with certainty, we feel that circumstantial evidence does not support it.

One other feature of the KIE puzzles us. The ¹⁸O and 4-¹⁴C KIE decrease with increasing conversion. This feature was not changed when the KIE were calculated with the use of conversions (*F₁*) computed with eq 1 for bifurcation of a reaction into two

$$F_1 = (1 - F_s)F / (1 - F_sF) \quad (1)$$

pathways (Scheme I), according to Melander and Saunders.²⁴ In this equation, *F_s* = 0.24 and *F* is the kinetic conversion listed in Table I. Therefore, we cannot explain the drift toward lower KIE, but there is no doubt that the KIE themselves are consistent with a concerted process.

2-¹⁴C KIE. The formation of byproducts having a carbazole structure offered the possibility that a second, [3,3]-sigmatropic rearrangement of **1** had occurred. Further, these products also introduced the possibility, noted earlier by Miller,⁶ that the major product **2** could be formed also by a sequence of two consecutive [3,3]-sigmatropic shifts. We decided to test these possibilities by measuring the KIE for forming both **2** and the major byproduct **9** from **1**, which was labeled in the 2'-position. For this work it was necessary to separate **2** by column chromatography first and then, by a second pass through a column, to separate **9** from other products and, in low conversions, also from unrearranged **1**. In order to have enough of **9** for isolation, it was necessary to restrict our low conversions to no less than 30%.

The KIE results are given in Table II. The KIE for formation of **9** have been adjusted for intramolecular competition, because, statistically, only one of the two ortho positions in the anilino ring of **1** is likely to be labeled with ¹⁴C. The results show that there is a large KIE in the formation of **9**. We conclude from the results that a [3,3]-sigmatropic rearrangement occurs in that pathway. This is illustrated with the formation of intermediate **14** in Scheme I. All of the other byproducts should, according to our scheme, show this KIE, too.

The KIE in the formation of **2** from [2-¹⁴C]-**1** appears to be an inverse one. Had **2** been formed solely by a sequence of two [3,3]-sigmatropic rearrangements, they should have given rise to a substantial KIE. Our data suggest, in fact, that none of **2** is formed by this pathway and that proton loss from the first [3,3]-intermediate (**14**) is too fast to allow for a second [3,3]-sigmatropic shift. In principle, the direct formation of **2** from [2-¹⁴C]-**1** should not show a KIE because the bonding carbon atom was not labeled. The inverse KIE, which we have measured, may be caused by errors in our measurements (that is the KIE should be 1.000) or may be an artifact because there is such a large KIE

(21) Kurz, J. L.; Daniels, M. W.; Cook, K. S.; Nasr, M. M. *J. Phys. Chem.* **1986**, *90*, 5357.

(22) Kurz, J. L.; Pantano, J. E.; Wright, D. R.; Nasr, M. M. *J. Phys. Chem.* **1986**, *90*, 5360.

(23) Tanaka, N.; Atsumi, Y.; Araki, M.; Kimata, K. *J. Am. Chem. Soc.* **1985**, *107*, 7781.

(24) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley-Interscience: New York, 1980; p 272.

(20) Yamataka, H.; Nagase, S.; Ando, T.; Hanafusa, T. *J. Am. Chem. Soc.* **1986**, *108*, 601.

in forming **9**. The inverse KIE for **2** says that the $^{12}\text{C}/^{14}\text{C}$ ratio in **2** early in the rearrangement was smaller than the ratio at the completion of the rearrangement. Early in rearrangement the ^{12}C content of **1** is depleted by the KIE of forming **9**. Consequently, this depletion will show up in product **2** and may have given rise eventually to an apparent inverse KIE for forming **2**.

Conclusions

The acid-catalyzed rearrangements of the quinamine **1** appear to occur by concurrent concerted processes. The major process is a [5,5]-sigmatropic rearrangement giving the product **2**. There is a minor process, a [3,3]-sigmatropic rearrangement, from which a number of byproducts are derived.

Fries reported two major classes of quinamine rearrangement, giving, respectively, diphenyl ether type and biphenyl type products. These are illustrated with the structures **2** and **4**. On the basis of our results with the prototype quinamine **1**, these two major types would appear to be [5,5]- and [3,3]-sigmatropic rearrangements, respectively. Fries reported, also, a less common type of quinamine rearrangement, the formation of a diphenylamine-based product, e.g., **6**. The mechanism of this rearrangement remains unclarified.

Finally, we turn again to the analogy between the quinamine and benzidine rearrangements. The acid-catalyzed rearrangement of hydrazobenzene gives two major products, benzidine and diphenylene. They are formed intramolecularly in the ratio of ca. 70:30. Benzidine formation is a [5,5]-sigmatropic rearrangement, in accordance with which there are substantial ^{13}C and ^{14}C KIE for carbon bonding.¹⁴ Diphenylene formation is formally a [3,5]-sigmatropic shift and cannot be suprafacially concerted according to the principles of conservation of orbital symmetry. Accordingly, diphenylene formation does not exhibit carbon-bonding ^{14}C and ^{13}C KIE.^{14,25} Here, then, the bifurcation is to concerted and nonconcerted rearrangement pathways, pathways that probably differ only little in energetics but materially in type. The action of acid on quinamine **1** also causes a bifurcation, but this time to two concerted pathways, from one of which **2** results and from the other of which a number of products derives.

Experimental Section

2,4-Dibromo-4,6-dimethylcyclohexadienone (13). 2-Bromo-4,6-dimethylphenol (**12**) was prepared in 64% yield by the bromination of 2,4-xyleneol as described earlier,²⁶ bp 136–138 °C (36 mmHg). Via essentially the procedure of Fries and Oehmke,³ a mixture of 15 g (75 mmol) of **12** and 12.3 g (150 mmol) of sodium acetate in 20 mL of acetic acid was cooled to –5 °C. At that temperature the mixture was solid and was powdered by crushing with a rod. The powder was stirred for 10 min with a solution of 12 g (75 mmol) of bromine in 10 mL of acetic acid, while the temperature was kept below 0 °C. During that time the bromine disappeared, and a thick, yellow paste was formed. The paste was filtered, and the residue in the funnel was washed with 10 mL of acetic acid, several times with water, with 5% sodium bicarbonate solution, again with water, and finally with 20 mL of cold ethanol. After drying in air for 1 h, the fine, yellow crystals of **13** (18.5 g, 88%) had mp 51–53 °C (lit.³ mp 59 °C): ^1H NMR (CDCl_3) δ 7.40 (m, 1 H), 6.80 (m, 1 H), 1.95 (2 s, 6 H, CH_3). In other preparations of **13**, yields of 74–97% were obtained.

As reported by Fries and Oehmke,³ **13** decomposes on standing and in attempts to crystallize it. We found that on standing between 1 and 5 h, depending on its purity, **13** decomposed exothermically, became red, and melted, only to resolidify as 2-bromo-4-(bromomethyl)-6-methylphenol. Decomposition of **13** occurred also if it was kept too long in the NMR solvent CDCl_3 .

An attempt was made to prepare **13** by direct bromination of 2,4-dimethylphenol in acetic acid, that is, without the isolation of intermediate **12**. A solution of 8 g (66 mmol) of 2,4-dimethylphenol and 11 g (135 mmol) of sodium acetate in 60 mL of acetic acid was cooled to below 10 °C. A solution of 21 g (132 mmol) of bromine in 40 mL of acetic acid was added dropwise with stirring. The mixture was kept overnight and diluted with 1 L of water. An oil separated and quickly solidified. The yellow-brown solid (6.5 g, 33%) was crystallized from petroleum ether and was found to be 2-bromo-4-(bromomethyl)-6-

methylphenol, mp 98–100 °C (lit.³ mp 104 °C).

Preparation of the Quinamine (1). The procedure of Fries and Oehmke was followed with one exception: the use of sodium acetate as base instead of an excess of aniline. This procedure was adopted in order to conserve [^{15}N]aniline in the synthesis of labeled **1**. In the event, it also turned out that higher yields of **1** were obtained when sodium acetate was used in place of an excess of aniline.

A mixture of 9.3 g (33 mmol) of freshly prepared **13**, 2.2 g (27 mmol) of sodium acetate, and 12 mL of ethanol was cooled to –5 °C. A solution of 3 g (32 mmol) of aniline in 3 mL of ethanol was added dropwise to the stirred, cold mixture, so as to keep the temperature below 0 °C. The mixture was stirred for 15 min after addition was finished, and then diluted with 10 mL of water. The product precipitated, was filtered, and was washed with 10 mL of water and 15 mL of cold ethanol. The yellow-brown product was dried in air, giving 6.1 g (66%). The crude quinamine was purified, with considerable loss, by dissolving in a mixture of 30 mL of acetone and 10 mL of ethanol and adding 10 mL of water to the filtered solution. The precipitated **1** was collected, washed with 10 mL of cold ethanol, and dried in air, giving 2.5 g (27%), mp 104 °C (lit. mp 107 °C,² 104–105 °C⁵): ^1H NMR (CDCl_3) δ 7.44–6.40 (m, 7 H), 4.0 (br s, 1 H, NH), 1.97 and 1.57 (2 s, 3 H each, CH_3).

Preparation of 2,4-Dimethyl[^{18}O]phenol. 2,4-Dimethylaniline was diazotized in fluoboric acid solution in the usual way, giving the solid diazonium tetrafluoroborate in 56% yield. Five grams of H_2^{18}O (50% ^{18}O) was mixed with 20 g of water and 6.3 g of concentrated sulfuric acid. To this solution was added 18 g (82 mmol) of the diazonium tetrafluoroborate, and the mixture was heated slowly to 50 °C, whereupon decomposition began. After evolution of nitrogen had stopped, the solution was heated at 60 °C for 30 min and cooled. An oil separated and was extracted with 2 \times 30 mL of methylene chloride. The aqueous layer was used again with a second 18-g portion of the diazonium tetrafluoroborate. The two sequences gave 19 g of product. The crude product was shaken with 100 mL of 2 M sodium hydroxide solution and 50 mL of benzene. The aqueous layer was acidified with 2 M hydrochloric acid and extracted with methylene chloride. Workup gave 9.5 g (48%) of labeled xylenol as an oil, calculated to contain 8 mol % of ^{18}O . The product was found, by TLC and ^1H NMR analyses, to be of satisfactory purity for conversion into [^{18}O]-**1**. After acid-catalyzed rearrangement of the [^{18}O]-**1**, the product (**2**) was found by mass spectrometry to contain ca. 8 mol % of ^{18}O .

[4- ^{14}C]Aniline was prepared by deaminating commercial (American Hoechst, Frankfurt) 4-nitro[1- ^{14}C]aniline¹⁴ and reducing²⁷ the nitrobenzene so obtained. The final product, after appropriate dilutions, had a specific activity of ca. 8 mCi/mol.

[2- ^{14}C]Aniline was prepared by analogous reduction of [2- ^{14}C]nitrobenzene. The product after appropriate dilution had a specific activity of 8 mCi/mol.

Preparations of labeled **1** were carried out with [^{15}N]aniline (commercial, 99% ^{15}N), [2- ^{14}C]aniline, [4- ^{14}C]aniline, and 2,4-dimethyl-[^{18}O]phenol as described for unlabeled **1**. The substrate used for measuring ^{15}N KIE contained 10 mol % of [^{15}N]-**1**.

Product (2) of Rearrangement of **1. Conditions of Fries and Oehmke.³** To a solution of 1.27 g (4.33 mmol) of **1** in 5.0 mL of acetic acid was added 1.2 mL of concentrated hydrochloric acid. After several minutes the mixture had so much precipitate as to appear to be solid. The mixture was heated in a bath of boiling water for 20 min and cooled. The large precipitate was collected, washed with 5 mL of water, and dried, giving 931 mg (65.4% as 2-HCl). A 746-mg portion was treated with 10 mL of 10% potassium bicarbonate solution. The product was extracted with methylene chloride (2 \times 50 mL) and worked up to give 628 mg (95% recovery) of **2**, mp 76–78 °C (hexane). TLC showed the crude product to be essentially free of other components.

Products of Rearrangement of **1. Miller's Conditions.** Miller studied the kinetics but not the products of rearrangement of **1**.⁶ The concentrations he used for the kinetics of rearrangements of quinamines in aqueous methanol were (1.0–5.0) $\times 10^{-5}$ M quinamine and (2.0–10.0) $\times 10^{-4}$ M hydrochloric acid. These concentrations were not suitable for our use, since they would not have given us enough isolated product for measurements of isotopic abundances. Therefore, we used concentrations similar to but not identical with those which Miller used for isolating products in a "crossing" experiment, designed to show the intramolecularity of rearrangements into diphenyl ethers. No byproducts were found in those rearrangements. Miller's concentrations were 6.0×10^{-2} M quinamine and 3.6×10^{-1} M hydrochloric acid. The concentrations we used were similar to these, but we used Miller's rate constant for the rearrangement of **1** under kinetic conditions to guide us in quenching the rearrangement after partial conversions for KIE measurements.

(25) Kupeczyk-Subotkowska, L.; Shine, H. J.; Subotkowski, W.; Zygmunt, J. *Gazz. Chim. Ital.* **1987**, *117*, 513.

(26) Orton, K. J.; Coates, J.; Burdett, F. J. *Chem. Soc.* **1907**, 91, 54.

(27) Vogel, A. *Textbook of Practical Organic Chemistry*, 4th ed.; Longman: London, 1978; p 659.

Rearrangements of **1** and workup of products were carried out in several ways, each designed for a particular objective. First, we describe the procedure for isolating **2**, identifying byproducts in groups by GC/MS, and isolating and identifying **8**. Then we describe in succession the procedure used for isolating only **2** for ^{18}O , ^{15}N , and ^{14}C KIE measurements, the procedure used for isolating both **2** and **9** for ^{14}C KIE measurements, and the procedure for isolating **9** for identification by NMR spectroscopy.

(a) Isolation of 2 and Identification of Byproducts. The concentrations of reactants were 8×10^{-2} M **1** and 2×10^{-1} M hydrochloric acid. To a stirred solution of 584 mg (2 mmol) of **1** in 22.5 mL of methanol was added 2.5 mL of 2 M hydrochloric acid. After standing for 24 h at room temperature, the solution was taken to dryness at 30–35 °C in a rotary evaporator, giving 597 mg of dark solid. The solid was treated with 30 mL of 2.5% sodium bicarbonate solution, and this was extracted with 2×30 mL of methylene chloride. Workup gave 539 mg of brown solid, shown by TLC to be composed of a number of compounds, the major being the diphenyl ether **2**. A 316-mg portion of the mixture was chromatographed on a column of silica gel (Baker, 3405R, 60–200 mesh) with methylene chloride/petroleum ether (2:1) as eluent. Aliquots of eluate were monitored by TLC and collected into three major fractions. The first fraction (I), 38 mg (12% of the mixture), mp 88–90 °C, was shown by GC to consist of two main and a few minor components. The larger of the main components (70% of I) was identified by GC/MS as a monochloro derivative (**8**) of 1,3-dimethylcarbazole: MS, *m/e* (relative intensity) 229 (M^+ , 100), 231 (M^+ , 31.7), 214 (15.4), 216 (5.5), 194 (87), 193 (15.6), 195 (13.1), 192 (18.4), 191 (16.9). The smaller component (10% of I) was identified to be 1,3-dimethylcarbazole itself (**7**): *m/e* (relative intensity) 196 (18.2), 195 (M^+ , 100), 194 (55.3), 180 (52.1). Compound **8** was separated from fraction I for NMR as follows. Fifteen milligrams of fraction I, dissolved in 2 mL of methylene chloride, was streaked on a preparative, 2-mm thick-layer plate of silica gel (EM 5717-7) and developed with petroleum ether. The major band was removed from the plate with methylene chloride to give 10 mg of an oil, which solidified on standing. Crystallization from hexane gave mp 94–96 °C: ^1H NMR (CDCl_3) δ 2.47 (s, 3 H, CH_3), 2.48 (s, 3 H, CH_3), 7.07 (s, 1 H, $\text{C}_2\text{-H}$), 7.24 (m, 1 H, $\text{C}_6\text{-H}$), 7.41 (m, 2 H, $\text{C}_7\text{-H}$ and $\text{C}_8\text{-H}$), 8.61 (d, 1 H, $J = 8.02$ and 0.66 Hz). Assignments of peaks were made with the help of data for compound **9**, which are given later.

The second chromatography fraction (II), 24 mg (7.6% of the mixture), mp 125–127 °C, was found by GC to consist of a major (94% of II) and two minor (4% and 2%) components. The major component was identified by GC/MS to be a monomethoxy derivative (**9**) of 1,3-dimethylcarbazole: MS, *m/e* (relative intensity) 226 (18.6), 225 (M^+ , 100), 211 (17.8), 210 (100), 182 (65), 180 (26.5), 167 (51.8). The 4% component appeared to be a dibromo derivative (**11**) of 2'-amino-3,5-dimethyl-2-hydroxybiphenyl (**17**): MS, *m/e* (relative intensity) 373 (61), 372 (33), 371 (100), 370 (17.6), 369 (56), 292 (1.4), 290 (2.2), 211 (45.5). The 2% component was identified to be a monobromo derivative (**10**) of 1,3-dimethylcarbazole: MS, *m/e* (relative intensity) 306 (19.3), 305 (M^+ , 99), 303 (M^+ , 100), 291 (10.4), 290 (43), 289 (10), 288 (42), 260 (10.3), 219 (23), 210 (11.6), 209 (57.5), 181 (23.8), 180 (20.4), 131 (17.7).

The third chromatography fraction (III), 255 mg (80.4% of the mixture), was shown to be **2**: mp 77–78 °C (from hexane); MS, *m/e* (M^+ , 292), ^1H NMR (CCl_4) δ 7.14 (s, 1 H), 6.80 (s, 1 H), 6.47 (s, 4 H), 3.26 (s, 2 H, NH_2), 2.26 and 2.10 (2 s, each 3 H, CH_3).

The major products of rearrangement were, thus, **2** (74.5%), **8** (10.0%), and **9** (8.7%). Here, **8** and **9** were formed in almost equal amounts. We found, however, as is shown below, that the relative amounts of **8** and **9** that were formed depended on the concentration of HCl in the rearrangement solution.

(b) Rearrangement of [^{18}O]-1**, [^{15}N]-**1**, and [^{14}C]-**1** for KIE Measurements.** In order to isolate enough of **2** for KIE measurements at low conversions without working with impractically high volumes of solution and yet keep concentrations as low as possible, we chose to work with 2×10^{-3} M **1**. An acidity of 6×10^{-3} M hydrochloric acid was chosen as being compatible and suitable for a reasonable rate of rearrangement under pseudo-first-order conditions, for which the rate constant, needed for timing low conversions, $0.126 \text{ L mol}^{-1} \text{ s}^{-1}$, was calculated from Miller's data at 26 °C.⁶ All rearrangements were carried out in aqueous methanol at 25 °C. An example follows.

A solution of 877 mg (3.0 mmol) of **1** (appropriately labeled) in 750 mL of methanol was kept at 25 °C. A second solution, consisting of 4.5 mL of 2 M hydrochloric acid (9.0 mmol) and 145.5 mL of water in 600 mL of methanol, also at 25 °C, was added rapidly to the first solution. The resulting concentration of **1** and acid were 2×10^{-3} and 6×10^{-3} M, respectively. Immediately a 500-mL portion (B) of the mixed solutions was removed, put aside for 2.5 h, and then made alkaline with 5 mL of 1 M sodium hydroxide. This solution (B) constituted the 100% con-

version sample. The major, 1000-mL portion was made alkaline with 10 mL of 1 M sodium hydroxide 293 s after mixing. The conversion of **1** in this solution (A) was estimated from the rate constant to be 20%.

Solution A was concentrated at 30–35 °C to 50 mL in a rotary evaporator. The concentrate was extracted with 3×50 mL of methylene chloride to give 537 mg (92% recovery, based on **1**) of a mixture of unrearranged **1** and products. All of the mixture was chromatographed on a column of silica gel with methylene chloride as eluent. Aliquots of eluate were monitored by TLC and collected to give two fractions: 444 mg of a mixture of **1** and byproducts and 80 mg of **2**. Thus, 524 mg (97.5%) of the crude mixture was recovered, of which 15.3% was **2**. This does not represent the total conversion of **1** since, as shown earlier and as will be seen below, rearrangement of **1** occurred into other products beside **2**.

Solution B was treated in the same way, giving 289 mg (99 wt % recovery, based on **1**) of solid. Chromatography gave 53.6 mg of a mixture of byproducts and 187 mg of **2**. The recovery of products was thus 83%, and the percent weight ratio of **2** to byproducts was 78:22.

Altogether, this procedure was repeated 14 times. Three of the runs were controls with unenriched **1**. The others gave pairs of samples of **2** from low and 100% conversions. In this way we obtained three pairs for ^{15}N , four pairs for ^{18}O , and four pairs for ^{14}C KIE measurements. The recovery of the crude mixture of products from the alkaline solution averaged 95.4% from low and 95.5% from 100% conversions. Chromatographic separation caused an average weight loss of 4.8% from mixtures of low and 12.1% from mixtures of 100% conversion. The cause for the losses in chromatography was the retention of a brown band at the top of the silica gel column. This band was always larger in 100% than in low conversion rearrangements. It was observed that the longer the time of rearrangement, and, also, the more concentrated the acid, the greater was the amount of "tarry" product at the top of the column.

The weight ratio of **2** to byproducts averaged $76 \pm 2/24 \pm 2$ in the 100% conversions. This ratio could not be measured in the low conversions because unrearranged **1** and byproducts were collected together. The nature of the byproducts is reported above.

All of the **2** isolated from a rearrangement was converted into its trifluoroacetyl derivative. For example, 115 mg of potassium carbonate was added to a solution of 80 mg of **2** (0.274 mmol) in 30 mL of methylene chloride. To the stirred suspension was added dropwise 173 mg (0.824 mmol) of trifluoroacetic anhydride. The mixture was refluxed for 30 min, cooled, and filtered for evaporation to dryness, which gave 107 mg (0.276 mmol, 100%) of product. All samples of the trifluoroacetyl derivative of **2** were purified by sublimation, twice for mass-spectrometric and four times for scintillation-counting measurements.

(c) Rearrangements of [^{14}C]-1** for KIE Measurements on Products **2** and **9**.** Rearrangements were carried out in the same way as described in b above. However, because the objective was to isolate both **2** and byproduct **9** for KIE measurements, it was necessary to restrict low conversions to no less than 30%. In that way, only, was it possible to isolate enough of **9** for purification and scintillation counting. In order to isolate **9**, more extensive chromatography was needed than was used in the procedure for other KIE measurements. An example is given.

A solution of 1.169 g (4.0 mmol) of labeled **1** in 1.0 L of methanol and a solution of 6.0 mL of 2 M hydrochloric acid in 800 mL of ethanol and 194 mL of water, each at 25 °C, were mixed. The resulting concentrations of reactants were 2×10^{-3} M in **1** and 6×10^{-3} M in HCl. Immediately, 500 mL (B) of the mixture was set aside for 100% conversion. The remaining 1500 mL (A) was quenched with sodium hydroxide after 470 s, this being the calculated time for 30% conversion. Workup of the solutions A and B gave, respectively, 827 mg (94.4 wt %) and 262 mg (89.7 wt %) of solids. Chromatography of all of each solid was carried out on a column of silica gel with methylene chloride elution. Aliquots of 20 mL were collected, and their contents were monitored by TLC.

Fourteen fractions (aliquots) of the products of low conversion were collected and contained the following: fraction 1, 2.8 mg of **8**; fraction 2, 21.6 mg of a mixture of **8** and **9**; fraction 3, 127 mg of a mixture of **9** and **1**; fractions 4–7, 401 mg of **1**; fractions 8 and 9, 59 mg of a mixture of **1** and **11**; fractions 10–14, 169 mg (19.3% conversion of **1**) of **2**. Thus a total of 780 mg (94.3%) of the original mixture of products was recovered. Product **2** was converted into its *N*-trifluoroacetyl derivative for sublimation and counting as described before.

The mixture of **8** and **9** from fraction 2 was rechromatographed on silica gel with elution by methylene chloride/hexane (1:1) to give 9.4 mg (0.042 mmol, 1.4% conversion of **1**) of pure **9**. An attempt to trifluoroacetylate the product, so as to give more material for sublimation and counting, failed. The product was recovered unchanged. It was, then, sublimed twice for counting.

Fourteen fractions of the products of 100% conversion were collected, comprising 220 mg (84% recovery) of the initial mixture. From these,

analogously, 17 mg (0.076 mmol, 7.6% of **1**) of **9**, mp 131–132 °C after sublimation, and 181 mg (62% of **1**) of **2** were isolated.

It is evident that under these conditions, in which the concentration of hydrochloric acid was lower than that used in procedure b, above, more of **9** than of **8** was obtained.

(d) Rearrangement of Unlabeled **1 and Isolation of **9**.** The objective here was to obtain enough of **9** for structural identification by NMR spectroscopy. To a solution of 146 mg (0.5 mmol) of **1** in 225 mL of methanol at 25 °C was added a mixture of 25 mL of water and 0.75 mL of 2 M hydrochloric acid at 25 °C. The resulting concentrations were $(2.0 \text{ and } 6.0) \times 10^{-3} \text{ M}$, respectively. The mixture was neutralized after 3 h with 2 mL of 1 M sodium hydroxide and evaporated to dryness to give 138 mg of a dark oil. Chromatography on a column of silica gel, with methylene chloride elution, gave 28.2 mg of a mixture of byproducts and 101 mg of **2**. The mixture of byproducts was chromatographed again with a 1:1 mixture of methylene chloride/benzene. Fractions were monitored by TLC, and those fractions that contained only **9** were combined and evaporated to give 11 mg of **9**, mp 130–131 °C. Sublimation gave 10 mg of **9**, mp 134.5–135.5 °C.

KIE Measurements. Nitrogen and oxygen KIE were obtained from whole molecule-ion mass ratios, measured on the trifluoroacetyl derivative of **2**. A Hewlett-Packard Model 5995 mass spectrometer was used in its selected-ion-monitoring (SIM) mode. Samples were introduced into the mass spectrometer via the solid-sample inlet, by using the direct insertion probe. Samples were heated as required to maintain a constant source pressure of $8 \times 10^{-7} \text{ Torr}$. Data collection was achieved by monitoring at 70 eV the abundances of ions of m/e 389 and 390 for nitrogen and of m/e 389 and 391 for oxygen. The average dwell time was 50 ms/ion. Four or five runs of ca. 5000 scans were made for each sample. The data were normalized for 100% m/e 389 and then corrected for the abundances of appropriate ions (389/390 for nitrogen, 389/391 for oxygen) in the unenriched derivative of **2**. In this way the enrichment of the heavy ion (m/e 390 or 391) was determined in samples at each conversion. In each run the number of scans was analyzed statistically in 25 blocks of scans, and in each block the absolute abundances were averaged. Thus, 25 measurements of relative abundances of masses M and $M + 1$ for nitrogen, and of M and $M + 2$ for oxygen were obtained, and the results were treated statistically. KIE were then calculated with eq 2, in which F is the extent of low conversion (A, Table I), and R_p and

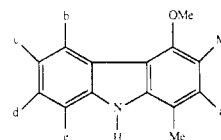
$$k_L/k_H = \ln(1 - F)/\ln(1 - R_p F/R_0) \quad (2)$$

R_0 are the isotopic ratios at low and 100% conversion, respectively.²⁸

Carbon KIE were obtained from ^{14}C abundances measured by scintillation counting with the use of a Beckman Model LS7000 liquid scintillation counter. Again, the trifluoroacetyl derivative of **2** was used. Three separate portions of each sample were weighed on a Cahn balance to $\pm 0.001 \text{ mg}$. In rearrangements of $[4\text{-}^{14}\text{C}]\text{-1}$, ca. 5.0-mg portions, and in rearrangements of $[2\text{-}^{14}\text{C}]\text{-1}$, ca. 1.0-mg portion were weighed. Each portion was counted 10 times and then averaged in counts/1.000 mg.

Thus, three measurements of counts/1.000 mg were obtained and averaged for calculating the KIE according to eq 2. KIE results obtained for rearrangements of $[2\text{-}^{14}\text{C}]\text{-1}$ were corrected for intramolecular competition, because only one ortho portion of **1** could be labeled.

Structures of **8 and **9**.** The structure of **9** was deduced to be 1,3-dimethyl-4-methoxycarbazole on the basis of COSY plots, ^1H NMR data in the literature, and the following 300-MHz ^1H NMR data (see structure **20**): ^1H NMR (CDCl_3) δ 2.44 (s, 3 H, CH_3), 2.47 (s, 3 H, CH_3), 3.96 (s, 3 H, OCH_3), 7.02 (s, 1 H, H_a), 7.24 (2 d, 1 H, H_c , $J = 7.62, 1.95$, and 6.84, 1.26 Hz), 7.40 (m, 2 H, H_d and H_e), 7.88 (br d s, 1 H, NH), 8.24 (d, 1 H, $J = 7.77 \text{ Hz}$). The small discrepancy in J_{cd} (7.62 Hz) and J_{de} (7.77 Hz) is attributed to the overlapping of peaks in the H_c multiplet.



20

On the basis of the mechanism of formation of byproducts, which is shown in Scheme 1, **9** could possibly be either the 2- or 4-methoxy derivative of 1,3-dimethylcarbazole. The reasoning here is that rapid deprotonation of **14** would occur before nucleophilic reaction. Therefore, nucleophilic reactions would take place only in the cyclohexadienone-like part of an intermediate, shown in Scheme 1 as **16**.

Sadtler ^1H NMR data for carbazole itself (Sadtler 6688 M) and a number of its derivatives (12104 M, 11622 M, 38250 M, 38249 M) show that the protons at C_4 and C_5 occur downfield from all others, in the region of 8.01–8.30 ppm, depending on structure and solvent.²⁹ Consequently, we should have for **9** either one (4-methoxy) or two (2-methoxy) downfield protons. Our data are consistent only with the 4-methoxy derivative. A COSY plot satisfied this structure, too, in that there was only one OCH_3/CH_3 interaction (instead of two expected for the 2-methoxy derivative), and there were two H/CH_3 interactions (for $\text{C}_2\text{-H}$) instead of one (expected for the $\text{C}_4\text{-H}$ in the 2-methoxy derivative). The COSY data were also inconsistent with the possibility that **9** was the 5-methoxy derivative.

By analogy with these ^1H NMR data, those of compound **8** are attributable to the 4-chloro derivative of 1,3-dimethylcarbazole.

Acknowledgment. We thank the National Science Foundation for generous support under Grant No. CHE 83-14947 and CHE 86-12031. This paper was written while H.J.S. was a guest in the Institut für Organische Chemie, Universität Hamburg. He thanks the faculty of the Institut for hospitality and the Alexander von Humboldt Foundation for an award.

(28) Reference 24, p 100.

(29) Sadtler Research Laboratories, Division of Bio-Rad Laboratories, Inc.