The Nitramine Rearrangement. Support of Nonconcertedness from Heavy-Atom Kinetic Isotope Effects

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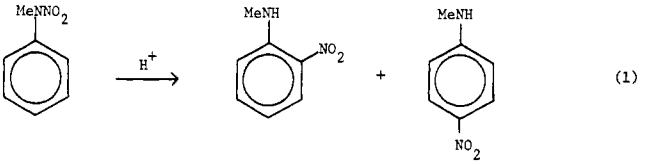
Abstract: Heavy-atom kinetic isotope effects (KIE) were measured for the formation of 2- (**2**) and 4-nitro-*N*-methylaniline (**3**) from the acid-catalyzed rearrangement of *N*-nitro-*N*-methylaniline (**1**). Separate rearrangements of [$^{15}NO_2$]-**1**, [2-^{14}C]-**1**, and [4-^{14}C]-**1** were carried out under kinetic conditions. Products **2** and **3** were isolated at 20%, 30%, and 100% conversions for nitrogen KIE measurements and at 20% and 100% conversions for carbon KIE measurements. Nitrogen KIE were determined from mass spectrometric measurements of the mass ratios M(153)/M(152) at low and 100% conversions. Carbon KIE were determined from scintillation count measurements of ^{14}C contents at 20% and 100% conversions. Substantial nitrogen KIE were found for the formation of **2** (average 1.0468) and **3** (average 1.0394). On the other hand a carbon KIE was not found for the formation of either **2** or **3**. These results suggest strongly that the nitramine rearrangement is not a concerted process and are consistent with White's dissociative, radical pathway.

N-Nitroarylamines undergo acid-catalyzed rearrangement into ring-C-nitro isomers.[2-4] The rearrangements are exemplified by that of *N*-methyl-*N*-nitroaniline (**1**), which rearranges into *o*- (**2**) and *p*-nitro-*N*-methylaniline (**3**, eq 1). The characteristics and

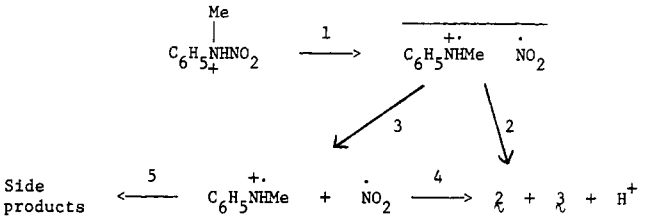


(1)

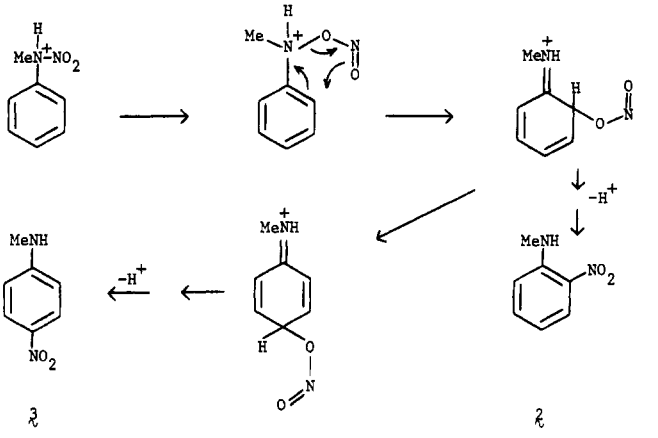
1 **2** **3**

generally accepted mechanism of the nitramine rearrangement have been deduced principally by White and co-workers, particularly from work with **1** and its derivatives. The rearrangements are specific acid catalyzed and first order in acid and in substrate. Rearrangement of **1** in aqueous acid solution is, in the main, intramolecular, although trapping experiments with hydroquinone have shown that there is also an intermolecular route to each isomer.[5-7] White has deduced from solvent, substituent, and trapping data that the rearrangement involves homolytic scission of the N–N bond of the protonated substrate followed by recombination of the fragments, an amine cation radical and the free radical, NO_2 (Scheme I). An earlier proposal for the mechanism of the nitramine rearrangement was made by Brownstein, et al., principally to account for the belief held then that rearrangement was entirely intramolecular.[8] This proposal has been called the "cartwheel" mechanism because its essential feature is the rearrangement of a postulated *N*-nitrito derivative in successive migrations analogous to those in Claisen rearrangements (Scheme II). Arguments against the validity of the "cartwheel" mechanism have been presented by White,[3] particularly as to the effects of ring substituents on the rearrangement of **1**. We do not wish to review the arguments for[9] or against the "cartwheel" mechanism, but note here only that it represents concerted breaking and making of bonds. The distinction between the two mechanisms, especially in the intramolecular formation of **2**, then becomes a distinction between concerted and nonconcerted rearrangements. In this case the concerted path is an allowed [3,3] sigmatropic shift. Formation of **2** by a direct [1,3] concerted, suprafacial migration of NO_2 from protonated **1** is, of course, not allowed. As for the formation of **3**, concerted migration is allowed, in principle, both by a direct route (a [1,5] shift) and by the "cartwheel" mechanism.

Scheme I



Scheme II



Recently, Ridd has presented evidence in support of White's mechanism with CIDNP effect data for the rearrangement of $^{15}NO_2$-labeled 2,6-dibromo-*N*-nitroaniline. Similar data were obtained for the rearrangement of $^{15}NO_2$-labeled **1**, but because the product composition was more complex, they were not pres-

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Table I. Nitrogen KIE ($k(^{14}N)/k(^{15}N)$) for Products **2** and **3** from the Rearrangement of [$^{15}NO_2$]-**1**

run	conversion, %	KIE, **2**	KIE, **3**
1	20	1.0439 ± 0.0008	1.0370 ± 0.0019
	30	1.0446 ± 0.0005	1.0373 ± 0.0002
2	20	1.0465 ± 0.0004	1.0437 ± 0.0011
	30	1.0521 ± 0.0012	1.0395 ± 0.0006

ented.[10] Ridd has pointed out that the CIDNP results are evidence for radical involvement and, thereby, support White's mechanism but has allowed also for the possibility that, in principle, the polarizations detected could be carried over in some other way from starting material which was reformed by the reverse of step 1.

Thus, conclusive distinction between concerted and nonconcerted paths of rearrangement has not yet been found, all evidence thus far having been only strongly indicative of the non-concerted path.

Results and Discussion

We have approached the question of concertedness by measuring heavy-atom kinetic isotope effects (KIE) in the rearrangements of [$^{15}NO_2$]-**1**, [2-^{14}C]-**1**, and [4-^{14}C]-**1**. The KIE were measured separately for the formation of **2** and **3** by isolating each isomer after rearrangement of a mixture of labeled and unlabeled **1**. Rearrangements were carried out in aqueous hydrochloric acid under kinetic conditions approximating those described by White.[5] Sampling was carried out at 20%, 30%, and 100% conversions for nitrogen and 30% and 100% conversions for carbon KIE. The KIE were then calculated from the isotope distribution in the products isolated at these conversions. Results are given in Tables I and II.

The nitrogen isotope data describe the timing of the breaking of the N–N bonds in protonated **1**. It is evident immediately from the results (Table I) that this bond is undergoing breaking in the transition state for product formation. We cannot read any mechanistic significance into the fact that the measured nitrogen KIE for forming **3** are a little lower than the KIE for forming **2**.

Rearrangements for obtaining carbon KIE were run most times without adding a radical trap, and therefore each of those KIE is a sum of the intra- and intermolecular rearrangements, in which, though, the intramolecular path is the larger. In one case with [2-^{14}C]-**1**, hydroquinone (HQ) was added as a trap at a concentration consistent with White's findings for complete quenching of the intermolecular portion of the rearrangement.[6] No other rearrangements in the presence of HQ were run because of the wastage of labeled substrate inherent in the trapping experiments and because of the internal consistency of the results at hand.

How do we assess the carbon KIE (Table II)? In effect, each of the two ^{14}C-labeled **1** isomers which we used has a built-in control or standard. For example, in the rearrangement of [4-^{14}C]-**1** there should be no KIE for the formation of the ortho isomer (**2**); that is, $k(^{12}C)/k(^{14}C)$ should be 1.000. Therefore our measured value of the KIE for the formation of **2** ($k(^{12}C)/k(^{14}C)$, average of runs 5 and 6, 1.0081) is a test of the precision of our measurements. In principle, the formation of **3** from [4-^{14}C]-**1** could have a sizable carbon KIE if a 1,5 sigmatropic shift occurred. Our measured value ($k(^{12}C)/k(^{14}C)$, average of runs 5 and 6, 1.0032) can be compared therefore with that for forming **2** from [4-^{14}C]-**1** to see if the measured value for **3** is significant. Our results show that there is no KIE for forming **3**, the result being, in fact, somewhat smaller than that for forming **2**.

The results from using [2-^{14}C]-**1**, if used without considering those from using [4-^{14}C]-**1**, could be ambiguous. That is, there should be no KIE for forming **2** by a direct 1,3 migration, unless, as is unreasonable, it were an antarafacial one. On the other hand, if the "cartwheel" mechanism were operating we could look upon the small KIE which was obtained ($k(^{12}C)/k(^{14}C)$, average of runs 1–3, 1.0052) for forming **2** as a valid measure of a concerted rearrangement and say, furthermore, that the KIE ($k(^{12}C)/k(^{14}C)$, average of runs 1–3, 1.0042) for going on to form **3** by a second [3,3] sigmatropic shift was fortuitously similar to that for forming **2**. However, there is no merit to this argument in view of the results from using [4-^{14}C]-**1**. That is, we regard the KIE of 1.0052 and 1.0081 for forming **2** from the two substrates as being the same and indicative of no isotope effect. The same conclusion applies also to the results for forming **3** from the two isotopes (averages: 1.0032 from [4-^{14}C]-**1** and 1.0042 from [2-^{14}C]-**1**).

The KIE from rearrangement in the presence of HQ (run 4) are a little higher than those measured in the absence of HQ. One might be tempted to argue that the data in run 4 reflect real intramolecular KIE and furthermore validate the "cartwheel" mechanism. However, there is, again, no merit in that argument when the data are compared with those from the rearrangement of [4-^{14}C]-**1**. That is, all of the measured KIE are of the same order of magnitude and tell us collectively that there are no carbon KIE in the nitramine rearrangement.

One aspect of our carbon KIE is difficult to explain. All of the averaged values are greater than 1.000, rather than being randomly around 1.000. Possibly, these results are real and associated in some way, as yet unclear, with a stepwise mechanism. We feel, rather, that there is a systematic fault in our measurements, but we are not certain of where it lies. It may be that the fault lies in our measuring of radioactivity in samples at low conversion. The average of errors in those measurements was a little higher (0.22%) than in measurements at 100% conversion (0.15%). The amount of product available for workup and purification at low conversion was always smaller than at 100% conversions and may have led to larger errors.

The summary of the data is, then, that there is a substantial nitrogen but no carbon KIE in this nitramine rearrangement. Although the absence of carbon KIE is not a conclusive proof, it is consistent with and suggestive of nonconcertedness. White's mechanism, therefore, for the two-step process, scission followed by recombination, is supported for the first time by direct probes of the bond-breaking and bond-making steps.

Experimental Section

***N*-Methyl-*N*-[^{15}N]nitroaniline ([$^{15}NO_2$]-1).** This compound was prepared by the reaction of *N*-methylaniline and labeled benzoyl nitrate.[11,12] The latter was made by adapting somewhat scant literature descriptions to our needs and used without isolation.[11,13,14] Silver [^{15}N]nitrate was made as follows. To a solution of 2.00 g (19.6 mmol) of $K^{15}NO_3$ (Stohler Isotope Chemicals 99% ^{15}N) in 10 mL of water was added a solution of 4.04 g (19.5 mmol) of anhydrous $AgClO_4$ (Apache Chemicals) in 10 mL of water. The mixture was allowed to stand for 1 h in the refrigerator, after which the precipitated $KClO_4$ was filtered and washed with 2 × 5 mL of ice–water. The combined filtrate and washings was evaporated and dried in a vacuum desiccator overnight, giving 3.17 g (96%) of $Ag^{15}NO_3$.

A suspension of the crude $Ag^{15}NO_3$ in 5 mL of methylene chloride, protected from moisture, was stirred at −15 °C. To it was added dropwise over a period of 5 min 2.38 g (17.0 mmol) of benzoyl chloride, and the resulting mixture was stirred for 3 h at −15 °C. Next, 10 mL of petroleum ether, precooled to 0 °C, was added, and the mixture was stirred again for 10 min, after which it was filtered directly into a solution of 1.81 g (17.0 mmol) of *N*-methylaniline in petroleum ether which had been precooled to −15 °C. Two 5-mL portions of cold petroleum ether were washed through the filtered AgCl into the reaction mixture. This mixture was stirred for 2 h at −15 °C and kept for 12 h at 0 °C in the refrigerator, after which it was filtered and evaporated at room temperature to give a solid orange-brown residue. The residue was dissolved in a small amount of benzene and chromatographed on a column of alumina (300 g, Woelm 04574, activity I) with benzene elution. Evaporation of the main fraction gave a yellow-orange oil which was dissolved in 80 mL of petroleum ether. Cooling in a dry ice–acetone bath gave 1.16 g (7.58 mmol, 38.6% based on KN^*O_3) of yellow solid. After four

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Table II. Carbon KIE ($k(^{12}C)/k(^{14}C)$) for Products **2** and **3** from the Rearrangements of [2-^{14}C]-**1** and [4-^{14}C]-**1**

run	KIE, **2**[b]	KIE, **3**[b]	run	KIE, **2**[c]	KIE, **3**[c]
1	1.0053 ± 0.0035	1.0054 ± 0.0018	5	1.0081 ± 0.0030	1.0016 ± 0.0007
2	1.0070 ± 0.0027	1.0050 ± 0.0035	6	1.0082 ± 0.0010	1.0081 ± 0.0048
3	1.0032 ± 0.0051	1.0023 ± 0.0027			
4[a]	1.0085 ± 0.0022	1.0071 ± 0.0019			

[a] Hydroquinone:substrate, 2.5 molar ratio. [b] From [2-^{14}C]-**1**. [c] From [4-^{14}C]-**1**.

crystallizations in the same way 450 mg (2.94 mmol. 15%) of colorless crystalline [$^{15}NO_2$]-**1** was obtained: mp 35.5–36.5 °C [lit. mp 36.6–37.6 °C].[15] For rearrangement and KIE measurements a mixture of 250 mg of [$^{15}NO_2$]-**1** and 4.75 g of **1** was crystallized from 500 mL of petroleum ether, giving 4.85 g of product, mp 35.5–37 °C, containing approximately 5 mol % of [$^{15}NO_2$]-**1**.

***N*-Methyl-*N*-nitro-[4-^{14}C]aniline ([4-^{14}C]-1).** [4-^{14}C]Nitrobenzene, approximately 8 mCi/mol, was available from earlier work.[16] Of this, 13.4 g (109 mmol) was stirred at 90–100 °C with 46.7 g (462 mmol) of triethylamine and 500 mg of 5% Pd/C while 16.5 g (348 mmol) of 97% formic acid was added over a period of 40 min.[17] The mixture was refluxed for 3 h, cooled, diluted with 100 mL of methylene chloride, and filtered. The filtrate was dried over $MgSO_4$ and evaporated to give crude, brown [4-^{14}C]aniline. Acetic formic anhydride was generated by adding 16.3 g (344 mmol) of 97% formic acid to 29.2 g (286 mmol) of acetic anhydride at 0 °C and heating at 40–55 °C for 2 h. After cooling, 15 mL of tetrahydrofuran (THF) was added; the solution was cooled to −15 °C and to it was added over a period of 20 min a solution of the crude [4-^{14}C]aniline in 30 mL of THF. After 1 h at 0 °C the mixture was evaporated to dryness on a rotary evaporator, and the brown oil (crude *N*-formyl-[4-^{14}C]aniline) so obtained was dissolved in 40 mL of THF. This solution was cooled to 0 °C and protected from moisture, and to it was added over a period of 40 min 27.0 mL (271 mmol) of borane–methyl sulfide complex (Aldrich), while the temperature of the mixture was kept at 0–5 °C. After the initial vigorous reaction ended the mixture was stirred 1 h at 20 °C and refluxed gently for 3 h. The mixture was cooled to 0 °C, diluted with 50 mL of methanol, and stirred for 1 h at 20 °C. Anhydrous HCl was bubbled into the mixture to pH 2, and the acidified solution was refluxed for 1 h. After 150 mL of methanol was added the mixture was evaporated to give a yellow oil. This was made basic with concentrated NaOH and extracted with 5 × 100 mL of ether. Evaporation of the dried ether extracts gave crude *N*-methyl-[4-^{14}C]-aniline. The product was nitrated (see above) to give, after four crystallizations, 4.50 g (29.6 mmol, 27%) of [4-^{14}C]-**1**; mp 35.5–37.0 °C.

***N*-Methyl-*N*-nitro-[2-^{14}C]aniline ([2-^{14}C]-1).** A mixture of 84 mg (2.58 mCi) of 2-nitro-[1-^{14}C]aniline (American Hoechst, 30.7 mCi/g) was diluted with 20.6 g (149 mmol) of 2-nitroaniline so as to give a ^{14}C content of 17 mCi/mmol. This mixture was deaminated in 100 mL of 50% H_3PO_2 by treatment at 0 °C with a small amount of Cu_2O and dropwise addition over 1.5 h of a solution of 12.4 g (180 mmol) of $NaNO_2$ in 40 mL of water. After the solution was made basic with 80 mL of 40% NaOH it was extracted with 4 × 200 mL of ether. Workup gave 11.9 g (96.7 mmol, 65%) of [2-^{14}C]nitrobenzene. A 7.5-g portion of this was diluted with 7.5 g of nitrobenzene, and the mixture was converted into [2-^{14}C]-**1** essentially as described for [4-^{14}C]-**1** and provided 7.50 g (49.3 mmol, 40.4%) of [2-^{14}C]-**1**; mp 35.5–36.5 °C, approximately 8 mCi/mol, after one crystallization from petroleum ether. Improvements over the preparation of the 4-^{14}C isomer were made in the procedure, however, by pumping off volatile byproducts from the crude *N*-formyl-[2-^{14}C]aniline with a vacuum pump and recovering crude *N*-methyl-[2-^{14}C]aniline not just by extraction with ether but by precipitation of the hydrochloride from the dried ether extract with HCl gas.

Rearrangements of Labeled 1. Rearrangements were carried out in aqueous 0.205 M HCl at 30 °C ± 0.5 following approximately the kinetic conditions of White.[5] Whereas White used approximately 10^{-4} M solutions of **1** for following its disappearance spectrophotometrically, we were obliged to use somewhat higher concentrations of **1** (3.3–7.9 × 10^{-3} M) in order to have enough of the isolated products for analysis. Since the rearrangement is first order in **1** we were able, nevertheless, to use White's rate constant for a given acidity. Times were calculated from the rate constant given[5] at which rearrangement could be stopped after 20% (27.5 min) and 30% (44 min) conversion by adding strong base. For 100% conversions the rearrangement solution was kept at room temperature for 48 h. In order to determine the nitrogen KIE, samples were isolated from rearrangements at 20%, 30%, and 100% conversions, whereas for the carbon KIE only 30% and 100% conversions were used. Products and unrearranged **1** were separated by flash chromatography and the products were purified by two recrystallizations. In the ^{14}C work each product was also sublimed after the crystallizations. The amount of **1** which was used for each run was 500 mg (3.3 mmol) in the nitrogen KIE case and 700 mg (4.6 mmol) in the carbon KIE case. Last, in the carbon KIE case carried out in the presence of hydroquinone 1.20 g (7.9 mmol) of **1** was used. The reason for using different amounts of **1** was to allow for enough product formation for isolation and purification. Further, when hydroquinone was used the yield of the products **2** and **3** was necessarily decreased. Rearrangements to the three conversions were carried out with separately weighed portions of **1**, again so as to allow for using a reasonable volume of the acid medium (1 L) and obtaining reasonable amounts of products for separation and purification. Typical examples follow.

Rearrangement of [$^{15}NO_2$]-1. A 2-L flask containing 1 L of 0.205 M aqueous HCl was thermostated at 30 °C ± 0.5. A solution of 500 mg of [$^{15}NO_2$]-**1** in 5 mL of dioxane was added quickly with rapid stirring. After 27.5 min 40 mL of 40% aqueous KOH was added. The yellow-orange solution was extracted with 4 × 200 mL of ether, and the ether extract was dried and evaporated to give 513 mg of a yellow oil. The oil was dissolved in 10 mL of toluene which was transferred into a 650-mL flash chromatography column (J. T. Baker) containing 20 cm of silica gel (Baker). Separation was effected with toluene at a flow rate of 2.5 cm/min. Fractions of 5 mL were collected, and each was monitored by TLC. The first 300 mL of eluent contained no dissolved material; the next 75 mL contained the ortho isomer (**2**); the following 100 mL contained a mixture of **1** and **2**. After that followed pure **1** and clean eluent (350 mL) and pure **3** (600 mL). **2** (51.8 mg of oil) was purified by crystallization twice from petroleum ether in a dry ice–acetone bath, while **3** (46.0 mg of solid) was crystallized twice from either toluene or benzene. Finally, 23 mg of **2**, mp 33–34 °C, and 14.7 mg of **3**, mp 150–151 °C, were obtained for mass spectrometry.

Rearrangement of [2-^{14}C]-1. Use of Hydroquinone. For rearrangement of [2-^{14}C]-**1** in the presence of hydroquinone (HQ) the HQ (2.17 g, 19.7 mmol) was dissolved in 1 L of aqueous 0.205 M HCl which was kept at 30 °C ± 0.5°. To this was added a solution of 1.20 g (7.9 mmol) of **1** in 12 mL of dioxane. The remaining procedure was the same as described earlier. Unused HQ and most of the benzoquinone (Q) formed in trapping reactions remained in the basic solution after quenching the rearrangement. Separation of the remaining HQ and Q from **1**, **2**, and **3** by flash chromatography was clean. As an example, from 20% conversion 40 mg of crude **2** (oil) and 36 mg of crude **3** (crystalline) were obtained. After crystallization and sublimation 10 mg of pure **2** and 8 mg of pure **3** were obtained for scintillation counting. Rearrangement of [4-^{14}C]-**1** in the presence of HQ was not carried out.

KIE Measurements. Nitrogen KIE values were calculated from the mass ratios (153/152) which in turn were determined mass spectrometrically on isolated samples of **2** and **3**. A Hewlett-Packard Model 5985B mass spectrometer was used. All samples were introduced into the mass spectrometer via the solid-sample inlet. Samples were heated as required to maintain a constant source pressure of 8 × 10^{-7} torr. Data collection was achieved by monitoring the absolute abundances of the 152 and 153 ions at 70 eV. A total of 19 950 repetitive scans per sample was obtained, with an average dwell time of 50 ms/ion. The resulting data were analyzed in blocks of 750 scans, the absolute abundances being added and then averaged for each block to yield the mean and its standard error, from which the ratio of M(153)/M(152) was calculated by routine statistical methods. The relative abundances of the M − 1 ion were negligible in compound **2** and small enough to be discounted in compound **3**. The kinetic isotope effects were then calculated[18] from these mass ratios determined at 20% and 100% and 30% and 100% conversions. The results are listed in Table I.

Carbon KIE were calculated from scintillation counting data, obtained with a Beckman LS 7000 scintillation counter and appropriate counting programs. Errors intrinsic to the counting were minimized by using a program with a 2σ error of ≤0.5% and making 10 separate counts for

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each assay of a sample. Weighing errors were minimized by using a Cahn balance and weighing 2.000-mg samples with an error of ±0.005 mg. A major problem in counting was the quenching effect of the colored products, amounting to almost 90% with **2** and 60% with **3**. Color quenching was eliminated by trifluoroacetylation, however. To do this, the 2.000-mg sample was placed in the counting vial, and 100 ± 0.1 μL of trifluoroacetic anhydride (TFA) was added. The vial was closed for 1 h at room temperature and then 10 mL of coctail (Packard SCINT-O) was pipetted into the vial. This gave a clear, colorless solution for counting. Trial experiments with standard samples and with **2** and **3** showed that this way of using TFA gave reasonably reproducibly results. Our overall practice then was to assay each of three separate 2.000-mg samples of each product according to the restrictions given above. An assay was made by making 10 timed, programmed counts, each count being of the order of 225 000. The 10 counts were then averaged, and the assays gave, eventually, three averaged counts for each sample of **2** and **3** at 30% and three averaged counts at 100% conversion. The deviation among the three averaged counts of samples at 30% conversion was often somewhat greater than the deviation among the three averaged counts at 100% conversion. Thus, the average of errors among all of the 30% conversion counts for **2** and **3** amounted to 0.22% while the average of errors among the 100% conversion counts was 0.15%. Four separate runs were made with [2-^{14}C]-**1** (one in the presence of HQ), and two were made with [4-^{14}C]-**1**. The calculated KIE are listed in Table II.

The data in Tables I and II list average errors in KIE. Calculations of the nitrogen KIE involve the terms $[(M + 1)/M]_{low}$ and $[(M + 1)/M]_{100}$ where low refers to 20% or 30% conversions and 100 refers to 100% conversion. The standard deviations in abundance measurements were used, therefore, to calculate a maximum and minimum measurement of an enriched (M + 1) abundance, normalized against 100% abundance for M and corrected for the natural abundance of (M + 1). Thus, for each conversion, low and 100%, we had, eventually, a maximum and minimum measure of the enrichment, mass (M + 1). Maximum and minimum KIE were calculated from these data for each run and expressed as an average in Table I. Calculations of carbon KIE also reflect the standard deviation in the average of three assays per sample. Thus, a maximum and minimum count was obtained for each sample at each conversion, and these led to a maximum and minimum KIE in each run, the average of which is expressed in Table II.

Registry No. [$^{15}NO_2$]-**1**, 90047-95-3; [2-^{14}C]-**1**, 90047-96-4; [4-^{14}C]-**1**, 90047-97-5; carbon-14, 14762-75-5; nitrogen-15, 14390-96-6.

Characterization of Molecular Aggregates of Peptide Amphiphiles and Kinetics of Dynamic Processes Performed by Single-Walled Vesicles

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Abstract: Peptide amphiphiles involving an L-alanine residue interposed between a charged head group and a double-chain segment, $N^+C_mAla2C_n$, were prepared, and the morphology of their aggregates in aqueous media was investigated by electron microscopy. Differential scanning calorimetry (DSC) was applied to examine thermodynamic properties of the bilayer aggregates associated with the phase transition between the gel and liquid-crystalline states. Appropriate lengths of the molecular skeleton ($n + m \leq 21$) and the double-chain segment ($12 \leq n \leq 16$) are required to obtain stable single-walled vesicles having the internal aqueous compartment under moderate sonication conditions. The exchange of amphiphile molecules between single-walled bilayer vesicles of a peptide amphiphile ($N^+C_5Ala2C_{16}$) and those of the corresponding spin-labeled amphiphile (SP-$N^+C_5Ala2C_{16}$) was investigated in a phosphate–borate buffer (pH 6.70, μ 0.10 with KCl) at 10.0–35.0 °C. The exchange took place via a collision mechanism rather than a diffusion-mediated process. The flip–flop behavior of the amphiphile molecules in the bilayer membrane, the permeability of sodium ascorbate into the vesicle, and the leakage of a water-soluble spin probe from the internal aqueous compartment of the vesicle were examined in the same medium. The single-walled vesicles thus prepared are stable enough to inhibit fusion under ordinary conditions.

Currently, there is growing interest in the physical properties and aggregation behavior of naturally occurring[1] and synthetic bilayer membranes.[2] Although the structures of phospholipid bilayers have been extensively investigated in connection with their biological functions,[3] their complexities and chemical instabilities have necessitated the development of more stable membrane-forming amphiphiles. Much effort has recently been exerted along this line, and a large number of membrane-forming amphiphiles have been prepared.[2] On the basis of electron microscopy, previous workers have claimed success in preparing several amphiphiles that form single-walled vesicles, having the internal aqueous compartment, without adding secondary components.[2a,j,4] We have recently shown that amphiphiles involving amino acid residues of various nature interposed between a polar head moiety and an aliphatic double-chain segment form distinct single-compartment vesicles in aqueous media, and such vesicles retain the morphology for a reasonably prolonged period of time.[5] In the light of the

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