

of D_2O . The reaction was started by adding 53 μL (660 μmol) of neat pyridine, which made the pH 4.4.

The loss of protons at C-5 was monitored by NMR and had a half-life of 25 min at 36 $^{\circ}\text{C}$, the temperature of the NMR probe. After 130 min, the reaction was stopped by adding 60 μL of 38% DCl (Norell), which dropped the pH below 2.

The mixture was applied to a 15.5 \times 1 cm diameter column of Dowex 50-X8 which had been equilibrated with purified 0.4 M HCl , made by diluting redistilled concentrated HCl . (The azeotropic distillate was titrated and found to be 6.97 M.) Elution was carried out at 2.4 mL/min with the same purified 0.4 M HCl while 2-min fractions were collected. The effluent was monitored continuously at 280 nm with an ISCO UA-5 monitor. The three major components of the mixture were well separated and eluted in the order ALA, pyridoxal, and pyridine, after the early elution of a small peak of absorbance which gave a negative test for amino groups with fluorescamine.⁸

The ALA fractions were pooled and evaporated under reduced pressure at room temperature, yielding 73.6 mg of ALA-5,5- d_2 hydrochloride (67%), mp 153–156.5 $^{\circ}\text{C}$ dec.⁹ The analysis of this material by NMR is described under Results and Discussion.

ALA-3,3,5,5- d_4 . In our hands, the base-catalyzed exchange procedure of Lester and Klein⁶ produced colored impurities which were difficult to remove. We found that acid-catalyzed exchange eliminated this difficulty.

ALA hydrochloride (2.46 g, 14.7 mmol) was refluxed in 40 mL of 15% DCl for 2.5 h. The solution was evaporated under reduced pressure at room temperature and the residual solid was dissolved in a fresh 30-mL portion of 20% DCl and then refluxed a further 2.5 h. A second evaporation gave a white crystalline solid, which was recrystallized by dissolving in 250 mL of boiling absolute ethanol and precipitating by chilling on ice and adding 300 mL of anhydrous ether. The yield of recrystallized ALA hydrochloride was low (1.43 g, 58%) due to solubility difficulties and esterification under these conditions.

NMR analysis of this material showed no signal above noise level at the positions of the H-3 and H-5 resonances.

ALA-3,3- d_2 . was prepared by the procedure described for ALA-5,5- d_2 above, using ALA-3,3,5,5- d_4 as starting material and H_2O instead of D_2O as solvent. The H-5 resonance in the NMR is far enough from HOD, even at 60 MHz, that its growth can be monitored in the H_2O solution. The reaction had a half-life of 38 min at 36 $^{\circ}\text{C}$; this retardation relative to the isotopically inverse reaction is the product of a deuterium kinetic isotope effect at the C-5 methylene and a solvent isotope effect.

NMR Analysis with EuCl_3 . For measurement with a high signal-to-noise ratio at 60 MHz, 17 mg (100 μmol) of a sample of ALA hydrochloride was dissolved in 340 μL of D_2O and neutralized with 1 equiv of NaOD (typically 40 μL of 2.5 M). The pH after such neutralization was 6.5. EuCl_3 (0.5 equiv) in D_2O was then added (typically 33 μL of 1.5 M) and the spectrum recorded. If the europium is added before the base, precipitation occurs.

Analogous procedures were used for the 90-MHz measurements at lower concentrations with EuCl_3 and PrCl_3 .

Acknowledgment. This work was supported in part by grant ES01572 from the United States Public Health Service. Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for Grant 3063-G1 in partial support of this research. We express our thanks to Dr. Stephen Marburg for helpful discussions.

Registry No. 2-HCl, 5451-09-2; 3-HCl, 75700-34-4; 4-HCl, 75700-35-5; 5-HCl, 75700-36-6.

(8) (a) Udenfriend, S.; Stein, S.; Böhnen, P.; Dairman, W.; Leimgruber, W.; Weigle, M. *Science* 1972, 178, 871. (b) Nakai, N.; Lai, C. Y.; Horecker, B. L. *Anal. Biochem.* 1974, 58, 563.

(9) Literature reports of the decomposition temperature of ALA hydrochloride vary considerably, e.g., 138–143 $^{\circ}\text{C}$,^{8a} 144–147 $^{\circ}\text{C}$,^{8a,c} 149–151 $^{\circ}\text{C}$,^{1,2d} and 156–158 $^{\circ}\text{C}$.¹⁰

(10) "Catalog Handbook of Fine Chemicals"; Aldrich Chemical Co.: Milwaukee, WI, 1980; p 57.

A Simple Procedure for the Analysis of Multisite Hydrogen-Deuterium Exchange Rates Obtained by Mass Spectrometry

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Received May 9, 1980

The measurement of hydrogen-deuterium exchange rates usually relies upon NMR or mass spectrometry. While the former technique allows the monitoring of specific hydrogen atoms undergoing exchange, it can be imprecise, especially when the substrate exhibits a complex spectrum. On the other hand, mass spectrometry yields very precise data on the isotopic composition of the substrate undergoing exchange, but it is frequently difficult to relate the results to the degree of reaction at any particular site. For example, consider the two-site exchange process in Scheme I where 1 bears diastereotopic protons H_x and H_y . If only d_0 material is present initially, then

$$[d_0] = [d_0]_0 e^{-k_1 t}$$

and

$$[d_1] = \frac{k_1 [d_0]_0}{k_2 - k_1} (e^{-k_1 t} + e^{-k_2 t})$$

Present interpretive methods^{1–3} which rely on equations such as those shown above suffer from the drawback that the basic exchange rate constants k_x and k_y are not easily available from k_1 and k_2 , except when $k_x = k_y$ and the exchange is statistically controlled.

An alternative and simple analysis lies in considering the progress of the reaction in terms of exchange at the specific sites themselves. Accordingly, if secondary isotope effects are neglected in Scheme I, then

$$[\text{H}_x] = [1] + [2] = [\text{H}_x]_0 e^{-k_1 t}$$

and

$$[\text{H}_y] = [1] = [3] = [\text{H}_y]_0 e^{-k_2 t}$$

Hence, if only d_0 material is present initially,

$$e^{-k_1 t} + e^{-k_2 t} = 2[1] + [2] + [3] = 2d_0 + d_1$$

where d_0 and d_1 are fractional isotopic species. Generalizing for n -site exchange, eq 1 is derived. For symmetrical

$$\sum_i^n e^{-k_i t} = \sum_i^n (n + 1 - i) d_{i-1} \quad (1)$$

substrates with p nonequivalent sites, the above equation reduces to eq 2.

$$\sum_i^p e^{-k_i t} = \sum_i^n (n + 1 - i) d_{i-1} \quad (2)$$

Equations 1 and 2 describe most exchange situations and may readily be incorporated into a simple and general computer program for routine use. We have written an iterative program⁴ based on Kim's procedure,⁵ and we have

(1) O. Hofer and E. L. Eliel, *J. Am. Chem. Soc.*, 95, 8045 (1973).

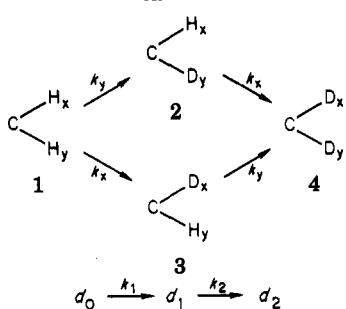
(2) J. Hine and W. S. Li, *J. Am. Chem. Soc.*, 97, 3550 (1975).

(3) N. M. Rodriguez and E. N. Rodriguez, "Consecutive Chemical Reactions", Van Nostrand, Princeton, NJ, 1964.

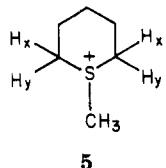
(4) See the Supplementary Material.

(5) N. Kim, *J. Chem. Educ.*, 47, 120 (1970).

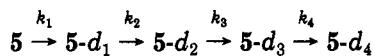
Scheme I



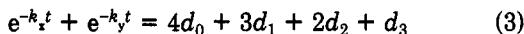
tested the program by using Eliel's data⁶ on H/D exchange in the sulfonium salt 5 which contains two pairs of dia-



stereotopic α -methylene hydrogens H_x and H_y . By treating the reaction according to the sequence



Eliel obtained $k_1, k_2, k_3, k_4 = 4.4:3.0:1.83:0.84$ from which he concluded that there was at best a very minor preference for abstraction of one proton pair over the other. Equation 2 for this particular case where $n = 4$ and $p = 2$ reduces to eq 3 from which we obtain rate constants of 1.83×10^{-2}



h^{-1} and $7.95 \times 10^{-3} h^{-1}$ for abstraction of the two sets of protons, i.e., a rate ratio of 2.3.

Finally we point out that the particular utility of eq 1 and 2 lies in their application to kinetics where the exchange rates differ by less than a factor of ten. In addition to examples such as the one cited above, this situation is likely to arise in isotope exchange of polycyclic homo- and heteroaromatic derivatives.

Acknowledgment. We thank the Natural Sciences and Engineering Council of Canada for financial support.

Registry No. 5, 41158-37-6.

Supplementary Material Available: A listing of the program (MULTISITE) written in BASIC (3 pages). Ordering information is given on any current masthead page.

(6) Obtained from the Supplementary Material to ref 1.

Hydride Abstraction from 9,10-Dihydroanthracene and 5,12-Dihydronaphthacene in an Aprotic Molten Salt Medium

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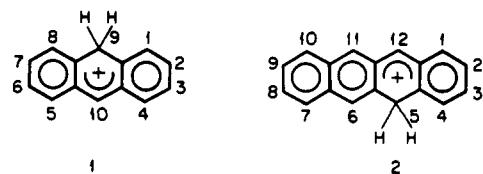
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We have recently reported several novel hydrogen-transfer reactions in molten antimony trichloride.^{1,2} For

example, anthracene in high-purity molten antimony trichloride undergoes a spontaneous catalytic disproportionation at temperatures as low as 100 °C.¹ Some of the anthracene molecules are condensed to larger aromatics, liberating hydrogen which is quantitatively captured by unreacted anthracene molecules to form hydroanthracenes. When 9,10-dihydroanthracene (DHA) is dissolved under the same conditions, it disproportionates to anthracene and 1,2,3,4-tetrahydroanthracene (THA). If the Lewis acidity and oxidizing power of the $SbCl_3$ solvent are increased by the addition of 10 mol % $AlCl_3$, the dissolution of anthracene in this melt results instead in the prompt formation of stable solutions of the anthracenium ion (1) in 55–65% yields.² The proton source for this reaction is the condensation–dehydrogenation of a portion of the anthracene combined with a stoichiometric reduction of $SbCl_3$.

We now report that DHA dissolved in high-purity, aprotic molten $SbCl_3$ –10 mol % $AlCl_3$ at temperatures from 100–130 °C also results in the prompt formation of 1. In this case, 1 is generated by a formal hydride abstraction from some of the molecules of DHA while the hydrogen abstracted is transferred to unreacted molecules of DHA to form a mixture of THA, 1,2,3,4,5,6,7,8-octahydroanthracene (OHA), and other hydroanthracenes and/or hydroarenes. The role of the Sb^{3+} as an oxidant is again evident in the recovery of stoichiometric amounts of Sb metal in the product mixtures. We have also found a similar reaction to occur for 5,12-dihydronaphthacene (DHN), producing the naphthacenium ion (2).



The formation of 1 and 2 was followed by 1H NMR measurements on the melts both with and without a $(CH_3)_4N^+$ internal standard. Their identities were confirmed by comparison of their respective integrated 1H NMR spectra with those obtained from authentic samples measured in various molten salt solvents.² The prominent feature in the spectra of these ions is the presence of a midfield peak for the hydrogens on the sp^3 -hybridized carbons at δ 4.9 with an integral twice that of the low-field-shifted resonance of H_9 (for 1) and H_{12} (for 2) at δ 9.6. The disappearance of the DHA starting material was observed by monitoring the resonance for its methylene hydrogens at δ 4.0. DHA was found to be completely reacted after 10–15 min at 100 °C and after less than 5 min at 130 °C. For DHN, the corresponding methylene resonance at δ 4.1 was not visible after the first few minutes even at 100 °C. At these times and temperatures, the arenium ions, 1 and 2, form in 70–80% yields and remain stable for periods of at least 2 h. In each case a mixture of hydroarene products appears as broad bands in the aliphatic region δ 1.7 to 3.0. In contrast, in neat $SbCl_3$ the hydrogen-transfer reactions of both anthracene and DHA proceed at a much slower rate such that the effects of time and temperature on the rate of the reaction could be followed for many hours.¹

(1) Dworkin, A. S.; Poutsma, M. L.; Brynestad, J.; Brown, L. L.; Gilpatrick, L. O.; Smith, G. P. *J. Am. Chem. Soc.* 1979, 101, 5299.

(2) (a) Buchanan, A. C., III; Dworkin, A. S.; Brynestad, J.; Gilpatrick, L. O.; Poutsma, M. L.; Smith, G. P. *J. Am. Chem. Soc.* 1979, 101, 5430.

(b) Buchanan, A. C., III; Dworkin, A. S.; Smith, G. P. *Ibid.* 1980, 102, 5262.