

Hydrogen Exchange of Aromatic Amines with D_2O and T_2O

BY B. J. FONTANA

Ingold, Raisin and Wilson¹ first demonstrated the ready H-exchange of the *o*- and *p*- H-atoms of phenols and aromatic amines when catalyzed by dilute bases and acids, respectively. This reaction was studied extensively by Kharasch, Brown and co-workers² in the case of the aromatic amines. It has been concluded that the existence of the quinoidal resonance forms of the free amine or phenolate ion is the chief factor governing the labilization of the nuclear H-atoms.

The amino or phenolic character of dyes or dye intermediates should then lend these substances amenable to the above type of H-exchange. Herein are described some quantitative exchange data for both deuterium and tritium obtained concurrently with attempts to label some organic dyestuffs with tritium. Discontinuance of the latter project has prevented the accumulation of more complete data.

Experimental

Exchange.—In general, 1 g. of the compound was mixed with 1 cc. of water (containing the D or T) and, unless otherwise noted, 0.01 cc. of 36 *N* sulfuric acid. The sealed mixture was heated at 100°, usually for seven days. The water was then distilled off *in vacuo* and purified by distillation *in vacuo* from potassium permanganate and sodium carbonate.

Analysis.—In all cases the change in the D or T content of the water was determined before and after exchange. The initial mole fraction of D was either 0.99 or about 0.7; the change in density observed, in general, was from 0.01 to 0.02 g. per cc. The method of density measurement is described elsewhere.³ Note that a 0.1% error in experimental results in a 10% error in the calculated exchange number in the case of deuterium.

The tritium⁴ as recovered from the 60-inch cyclotron had an activity of approximately 600 microcuries per mole of water (about 10^{-10} *M* T_2O) and contained 0.7 mole fraction of D. The D and T exchange could thus be determined simultaneously. In some experiments this water was diluted 1000-fold with H_2O . The very weak beta-radiation from H^3 , tritium,⁵ requires that the activities be determined by direct introduction of the hydrogen as a gas (water vapor) inside the tube of a Geiger counter. A mixture of 1-cm. pressure of water vapor and 2 cm. of pro-

pane was very satisfactory as a counting gas. The change in activity observed, in general, amounted to 20–30%.

Results and Discussion

The experimental results are tabulated in Table I. The observed values of the exchange number are given in the columns headed nkd and nkt . The number of exchangeable hydrogens, n , is merely the number of unoccupied nuclear positions *o*- and *p*- to the activating amino groups. That only the *o*- and *p*-positions undergo exchange has been assumed in general in H-exchange of this type, and has been shown to occur conclusively in the cases of phenol and aniline by Best and Wilson.⁶ The partition ratios follow, kD for deuterium and hydrogen; and kT for tritium and hydrogen, except in the cases of methylene blue and crystal violet where kT is an over-all partition ratio for T, D and H. The values of nk given have been corrected, where necessary, for exchange with added catalyst, amino hydrogens (assuming $k = 1.00$ for D or T), for water of crystallization in the case of methylene blue and for hydrochloric acid in the experiments with benzidine.

TABLE I
EXCHANGE NUMBERS AND PARTITION RATIOS

Expt.	Compound	n	nkd	kD	kD (cor.)	nkt	kT
1	Crystal violet	6	7.04	1.17	0.95
2			8.38	1.42	1.22	9.18	1.53 ^a
3	Methylene blue	4	4.14	1.04	0.85
4			4.84	1.21 ^b	0.98
5			5.65	1.41	1.22	5.21	1.30 ^a
6	Methyl orange	4	4.96	1.23 ^{c,d}	1.00
7	Congo red	8	3.78	0.47 ^d	0.38
8	Benzidine	8	1.72	0.22	0.18
9		2.86	0.33
10		0.90	0.11 ^{e,f}
11	Benzidine·1 HCl ^g	0.60	0.08 ^{f,h}
12	Benzidine·2HCl	2.90	0.36 ^h

^a Usual heating period plus six weeks at room temperature. ^b 0.03 cc. of 12 *N* hydrochloric acid as acid catalyst. ^c Extensive decomposition; value fortuitous? ^d Eleven days at 100°. ^e 0.02 cc. of sulfuric acid catalyst. ^f Solvent 50% ethyl alcohol (95%) and 50% water; ten days at 100°, three months at room temperature. ^g Mixture 50% benzidine and 50% benzidine·2HCl. ^h No added acid catalyst.

The kD values for methylene blue and crystal violet have been recalculated for zero D-content of water according to the theory of Brodskii.⁷ The average of the five corrected values gives $kD = 1.04$, which then might be compared to the values 0.90 and 1.4 for the nuclear hydrogens in phenol, obtained, respectively, by Ingold, *et al.*, and Small and Wolfenden.⁸ Partial exchange of

(1) C. K. Ingold, C. G. Raisin and C. L. Wilson, *J. Chem. Soc.*, 1637 (1936).

(2) M. S. Kharasch, W. G. Brown and J. McNab, *J. Org. Chem.*, **2**, 36 (1937); W. G. Brown, M. S. Kharasch and W. R. Sprowls, *ibid.*, **4**, 442 (1939).

(3) B. J. Fontana and M. Calvin, *Ind. Eng. Chem., Anal. Ed.*, **14**, 185 (1942).

(4) L. W. Alvarez and R. Cornog, *Phys. Rev.*, **56**, 613 (1939).

(5) S. C. Brown, *ibid.*, **59**, 954 (1941).

(6) A. P. Best and C. L. Wilson, *J. Chem. Soc.*, 28 (1938).

(7) A. I. Brodskii, *Trans. Faraday Soc.*, **33**, 1180 (1937).

(8) P. A. Small and J. H. Wolfenden, *J. Chem. Soc.*, 1811 (1936).

the meta hydrogens might account for the latter high value. Using the average value of $k_D = 1.2$ (uncorrected) and the values for the over-all k_T 's observed in experiments 2 and 5, the partition ratio for T and D is calculated to be approximately 1.2. The corresponding calculation for the k of T and H is very sensitive to experimental error; a value of approximately 2.0 is obtained, which seems very high. The apparent confirmation of this latter value by the results of experiments 8, 9 and 12 with benzidine is very probably fortuitous. The assumption that $k = 1.00$ for the exchange of T with amino H's is obviously questionable.

The triterated crystal violet lost only about 2% of its T-activity on standing in 2% aqueous solution for three months at room temperature. Heating this solution for seven days at 100°, with added sulfuric acid catalyst, caused a loss of 60% of the activity. The deuterated methylene blue (expt. 4) was heated with 1 cc. of water at 100° for two hours with no acid catalyst present. The calculated Δd for exchange of the 3H₂O of crystallization only was 0.0104, actually observed 0.0111. Such exchange was incomplete in only five minutes of heating or in twenty-four hours at room temperature.

As noted above, the values given for benzidine may not be valid. The calculated effect is a small one derived from the large observed effect due chiefly to exchange of the four amino H's. However, one interesting feature of the k values can be noted; that is, the very low values of the partition ratios in general. That these are real and not merely an indication of a slow rate of reaction is indicated first in experiment 10. Here, increased solubility was achieved by using a 50% aqueous ethyl alcohol mixture as the exchange medium. This is further substantiated by experiment 11. According to the theory and actual observations of Ingold, *et al.*,¹ on the exchange of phenol, and of Brown, *et al.*,⁹ on dimethylaniline, the conditions in expt. 11 are such as to yield the maximum possible rate of exchange. The result of expt. 12 as compared to expt. 9 is also consistent with the latter theory. The lower partition ratio observed in the aqueous alcohol mixtures can be explained in part by the observation of Kharasch, Brown, *et al.*,^{2,8} that k_D in pure ethyl alcohol as exchange medium is about 0.85 (as compared to approxi-

mately unity in water). The low value of k observed with benzidine seems to be in accordance with the theory of exchange activation by appearance of a negative charge on the ortho and para positions through the quinoidal resonance forms (of the free base in the case of aromatic amines). For in the case of benzidine there must be an interference between the resonances of the two groups with the aromatic nucleus, since the two resonance systems induce similar charges in the benzene rings. Such an effect does not seem to appear in the results obtained by Brown and Letang¹⁰ with various dimethylamino-naphthalene derivatives.

A similar resonance interaction would account for the relatively small exchange observed with the symmetrical dyestuff congo red. Accordingly, it was also ascertained that under the conditions employed herein, little or no exchange occurred in the dyes trypan blue and trypan red. Other effects may also be operating here, including the steric hindrance of resonance ("peri-effect") observed in naphthalene derivatives.⁹

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X-Ray and Optic Measurements on β -Lactoglobulin

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Through the courtesy of Dr. T. L. McMeekin, a large air-dried crystal of β -lactoglobulin was made available to the writer. In the only published work on this material,¹ no optic data (save the sign of the birefringence) and no correlation of the crystallographic axes with the physical directions of the crystal were given. It was thought desirable in view of McMeekin and Warner's² observations on shrinkage to make these measurements.

The crystals are of the orthorhombic variety designated by Crowfoot as tabular. The small-

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