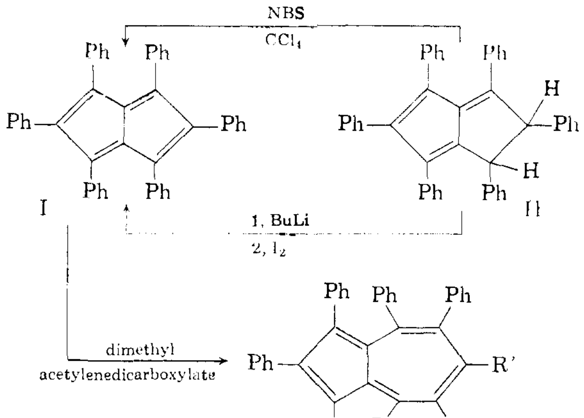

IIIa, R = Ph, R' = CO_2CH_3
b, R' = Ph, R = CO_2CH_3

deep blue with antimony pentachloride. Trifluoroacetic acid produces a deep blue color with I and a fluorescent red color with II. Addition of hydroxylic solvents immediately discharges these intense colors.

Heating I with excess dimethyl acetylenedicarboxylate at 160° affords in 63% yield the dicarbomethoxyhexaphenylazulene, III (a or b) [dark greenish-blue crystals, m.p. 247–248°; calcd. for $C_{50}H_{36}O_4$: C, 85.69; H, 5.18; mol. wt., 700.8. Found: C, 85.54; H, 5.10; mol. wt., 664. Ultraviolet-visible spectrum (chloroform) λ_{max} mμ (log ϵ): 265 (4.64), 339 (4.96), 634 (2.80). Infrared (KBr): 3.27, 3.39, 5.75, 6.24, 6.67, 6.91, 7.84, 8.17, 9.25, 9.71, 13.09, 14.32].[11]

Thus, the pentalene structure of I is consistent with: (1) the mode of formation (two routes), (2) the analytical results, (3) the unusual spectral properties, and (4) the unique two carbon ring-expansion to form an azulene.

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THE ISOTOPE EFFECT ON ACID-CATALYZED SLOW PROTON TRANSFER IN DILUTE AQUEOUS SOLUTION[1]

Sir:

Few examples of slow proton transfer from catalyzing acid to substrate are known, and not many measurements of the isotope effect on this reaction have been made. In the several cases reported so far, the deuterium isotope effect (k_H/k_D) has not been larger than two whenever the catalyzing acid has been the solvated proton in dilute aqueous solution.[2] Since this small isotope effect is in accord with at least one theoretical prediction,[3] the conclusion has been drawn that this isotope effect will always have a value near unity, and this value has been used to deduce the relative acid strengths of the species H_2O and D_2O.[2b] We wish here to report a deuterium isotope effect on rate-determining proton transfer from the solvated proton which is considerably greater than unity.

Acid-catalyzed aromatic hydrogen exchange in 1,3,5-trimethoxybenzene is known to proceed by the two-step reaction sequence[4]

$$\begin{aligned} &\mathrm{H'Ar} + \mathrm{H^+(H_2O)}_n \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{H'ArH^+} + n\mathrm{H_2O} \underset{k_1}{\overset{k_2}{\rightleftarrows}} \\ &\qquad\qquad\qquad\qquad \underset{\text{slow}}{\mathrm{HAr}} + \mathrm{H'^+(H_2O)} \qquad (1) \end{aligned}$$

The first forward step in this reaction is a slow proton transfer from catalyzing acid to substrate. The isotope effect on this step ($k_1^{H_2O}/k_1^{D_2O}$) as well as that on the second step (k_2^H/k_2^D) can be obtained by performing the experiments (I), (II) and (III).

$$\begin{aligned} &\text{I.}\quad \mathrm{TAr} + \mathrm{H^+(H_2O)}_n \rightleftarrows \mathrm{TArH^+} + n\mathrm{H_2O} \rightleftarrows \\ &\qquad\qquad\qquad \mathrm{HAr} + \mathrm{T^+(H_2O)}_n \qquad (2) \\ &\text{II.}\quad \mathrm{DAr} + \mathrm{H^+(H_2O)}_n \rightleftarrows \mathrm{DArH^+} + n\mathrm{H_2O} \rightleftarrows \\ &\qquad\qquad\qquad \mathrm{HAr} + \mathrm{D^+(H_2O)}_n \qquad (3) \\ &\text{III.}\quad \mathrm{TAr} + \mathrm{D^+(D_2O)}_n \rightleftarrows \mathrm{TArD^+} + n\mathrm{D_2O} \rightleftarrows \\ &\qquad\qquad\qquad \mathrm{DAr} + \mathrm{T^+(D_2O)}_n \qquad (4) \end{aligned}$$

These reactions are practically non-reversible, and their observed second order rate constants are related to the rate constants for the individual steps in the following way

$$k_{\text{obs}} = \frac{k_1}{1 + k_2^{\text{H}}/k_2^{\text{H}'}} \qquad (5)$$

With the aid of a relationship between deuterium and tritium isotope effects,[5] equations of the form of (5) for reactions I, II and III can be solved for

$$(k_{\text{obs}})_{\text{I}} = \frac{k_1^{\mathrm{H_2O}}}{1 + k_2^{\text{H}}/k_2^{\text{T}}} = \frac{k_1^{\mathrm{H_2O}}}{1 + (k_2^{\text{H}}/k_2^{\text{D}})^{1.442}} \qquad (6)$$

$$(k_{\text{obs}})_{\text{II}} = \frac{k_1^{\mathrm{H_2O}}}{1 + k_2^{\text{H}}/k_2^{\text{D}}} \qquad (7)$$

$$(k_{\text{obs}})_{\text{III}} = \frac{k_1^{\mathrm{D_2O}}}{1 + k_2^{\text{D}}/k_2^{\text{T}}} = \frac{k_1^{\mathrm{D_2O}}}{1 + (k_2^{\text{H}}/k_2^{\text{D}})^{0.442}} \qquad (8)$$

the rate ratios $k_1^{H_2O}/k_1^{D_2O}$ and k_2^H/k_2^D. (This treatment neglects secondary isotope effects in the aromatic molecule, but there are both empirical[6] and theoretical[5b,6a] reasons to expect these to be absent.) For 1,3,5-trimethoxybenzene in 0.05 *M* $HClO_4$, the data (Table I) give the values: $k_1^{H_2O}/k_1^{D_2O}$ = 2.93 σ = 0.07, and k_2^H/k_2^D = 6.68, σ = 0.18 (σ is the standard deviation of the mean value).

The isotope effect k_2^H/k_2^D is near the maximum value for C–H bond-breaking in a methylene group. The predicted rate ratio based on a simple consid-

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[1] Work supported by the Atomic Energy Commission, in part under USAEC Contract AT(11-1)-1025 and in part by Brookhaven National Laboratory.

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eration of zero-point energy differences for a C–H stretching vibration of 2900 cm.$^{-1}$ is 7.8, and the largest deuterium isotope effect reported in electrophilic aromatic substitution is 6.67.[7] Thus, the isotope effect weakening produced by the symmetrical stretching vibration in the transition state of a three-center reaction must be near its minimum value in this case.

The O–H stretching vibration in the solvated proton occurs at 2900 cm.$^{-1}$,[8] and the value predicted for the other isotope effect, $k_1^{H_2O}/k_1^{D_2O}$, is 7.8 as well. This is considerably greater than the observed value of 2.93. But the transition states of the two steps in the exchange reaction are the same, and, if the observed value of k_2^H/k_2^D is near its maximum value, the observed value of $k_1^{H_2O}/k_1^{D_2O}$ must be near its maximum value also. This discrepancy can be understood in terms of a predicted secondary effect of the water molecules solvating the proton.[9] When a proton is transferred from its solvent shell, the solvating water molecules revert to ordinary water. Since the O–H stretching vibration in liquid water is 3400 cm.$^{-1}$, this process is accompanied by considerable bond-tightening. It has been estimated that the deuterium isotope effect on this change is 0.7 per O–H bond for an equilibrium process,[9a] and the prediction has been made that this will reduce the kinetic isotope effect on proton transfer to a maximum value of about 3.6.[9b] The observed effect of 2.93 is in good agreement with this prediction.

TABLE I

RATES OF AROMATIC HYDROGEN EXCHANGE BETWEEN 1,3,5-TRIMETHOXYBENZENE AND 0.050 M $HClO_4$ AT 25°

Substrate	Solvent	$10^2 k_2$ (M^{-1} min.$^{-1}$)	No. of runs
TMB-t	H_2O	3.722 ± 0.030^a	9
TMB-d	H_2O	7.98 ± .10^a	7
TMB-t	D_2O	6.286 ± .012^a	5

[a] Error estimates are standard deviations of the mean values.

The difference between this approximately maximum isotope effect on proton transfer to 1,3,5-trimethoxybenzene and the other smaller isotope effects on slow proton transfer[2] is understandable in terms of the relative basicities of the various proton donors and acceptors.[9b] Isotope effects in three-center reactions will be less than their maximum value whenever the transition state is not truly symmetrical, that is, whenever the two force constants governing the symmetrical stretching vibration in the transition state are not equal.[10] In the slow proton transfer reactions on which isotope effects have heretofore been reported, the proton acceptor has usually been a strongly basic anion. In these transition states, therefore, the proton is not bound with equal strength to the acceptor molecule and the solvating water which it is leaving. Trimethoxybenzene, on the other hand, is a weaker base than these anions, and in this case the two forces holding the proton in the transition state should be more nearly equal. Thus, the isotope effect with trimethoxybenzene should be stronger than those reported before.

This isotope effect on proton transfer to trimethoxybenzene is twice the value which was used to estimate that H_2O is five times as strong an acid as D_2O.[2b] Similar reasoning with the data for trimethoxybenzene gives a ten-fold difference in acid strength between H_2O and D_2O. This unreasonably high isotope effect emphasizes the danger inherent in basing conclusions on the assumption that isotope effects will have essentially constant values.

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CELLULOSE COLUMNS CONTAINING POLYRIBONUCLEOTIDES AND RIBONUCLEIC ACIDS[1]

Sir:

Recent studies[2,3] have shown that polydeoxyribonucleotides can be covalently linked to cellulose and then be employed as chromatographic adsorbents which selectively bind polynucleotides complementary to them in base sequence. The methods of synthesis utilized the glucosidic hydroxyl groups of T4 DNA[2] and the terminal phosphate groups of thymidine oligonucleotides.[3] This suggested to us that the free hydroxyl groups on ribose C'_2 in polyribonucleotides might be available for similar reactions. We have found that phosphocellulose can indeed be linked to synthetic polyribonucleotides and made into columns capable of binding and desorbing polynucleotides. The specificity of adsorption with respect to the nature of the bases, salt concentration and temperature, is very similar to that for the formation of helical complexes in solution. Columns also have been prepared from natural ribonucleic acids. Use of these columns may constitute a chromatographic method for isolating cellular components complementary in base sequence to the RNAs.

We followed a procedure similar to Bautz and Hall's[2] adaptation of Khorana's carbodiimide reaction for forming phosphate-ester bonds between acetylated phosphocellulose (Serva, 0.78 meq. P/g.) and each of the listed polyribonucleotides: poly-A, poly-C, poly-I, poly-U, bacteriophage virus RNA, *E. coli* transfer and ribosomal RNAs. Nucleotide polymers and cellulose were dissolved in pyridine and reacted with dicyclohexyl carbodiimide at 115° for one hour. After the reaction product was isolated, it was chopped in a Waring blender at 4°, ground in a mortar, and washed extensively with neutral buffer at 80° to liberate pyridine and starting materials. After removal of

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[1] Abbreviations used: poly-A or simply A, polyriboadenylic acid; poly-C or C, polycytidylic acid; poly-I or I, polyinosinic acid; poly-U or U, polyuridylic acid; tris, tris-(hydroxymethyl)-aminomethane.

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