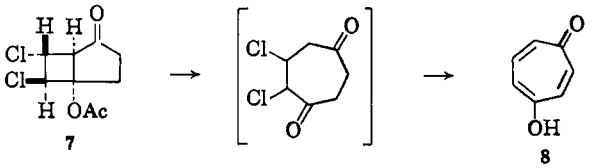
frequently, by such means as bromination–dehydrobromination. The following synthesis of γ-tropolone illustrates the point at issue.

Irradiation of the enol acetate of cyclopentane-1,3-dione in ethylene dichloride led to the production of three stereoisomers. One of these, m.p. 90–91°, provisionally allocated from n.m.r. data the stereochemistry shown in **7**, on standing at room temperature in methanol containing N,N-dimethylaniline (1%) was methanolized, in 36 hr., to the ketol which presumably underwent spontaneous dealdolization. Treatment of this, without isolation, with dilute aqueous sodium hydroxide[12] at room temperature for 3 hr. led to the elimination of the elements of hydrogen chloride and the formation of γ-tropolone (**8**).[13] The yield, estimated spectroscopically, was 45%.



7 8

Acknowledgment.—This work was supported by the U. S. Army under Grant DA-ARO(D)-31-124-G399.

(12) Omission of the methanolysis step led to quite different reactions which are presently being investigated.

(13) Identified by ultraviolet spectra in neutral and alkaline solution and by melting point.

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RECEIVED JULY 13, 1964

The Mechanism of the Acid-Catalyzed Isomerization of *cis*-Stilbene

Sir:

Following our recent studies[1,2] of the acid-catalyzed isomerization of *cis*-cinnamic acids, we have investigated the isomerization of *cis*-stilbene. *cis*-Stilbene is readily isomerized quantitatively to *trans*-stilbene in 50–60% sulfuric acid, as summarized in Table I. From 10 to

TABLE I

RATE OF ISOMERIZATION OF *cis*-STILBENE[a]

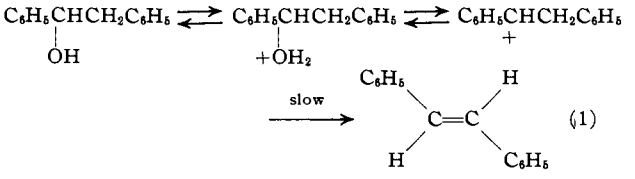
H_2SO_4			D_2SO_4	
Wt.%	H_0[b]	k, sec.$^{-1}$	Wt.%	k, sec.$^{-1}$
49.92	−3.40	1.28×10^{-5}		
55.50	−4.02	7.35×10^{-5}	52.58	1.99×10^{-5}
57.62	−4.28	1.43×10^{-4}	58.10	1.43×10^{-4}
60.92	−4.69	5.12×10^{-4}		
63.17	−4.95	1.10×10^{-3}	62.77	6.94×10^{-4}
66.06	−5.40	3.84×10^{-3}	65.45	2.37×10^{-3}

[a] The reaction medium contained 5% added ethanol to impart sufficient solubility to the stilbenes; initial concentration of *cis*-stilbene 2×10^{-6} *M*; reaction followed by ultraviolet spectroscopy using 10 cm. cells; *T* 25.00°. [b] The H_0 values were measured. A full discussion of the H_0 scale in 5% ethanol will be presented in a complete paper.

90% completion the reaction followed excellent first-order kinetics in any individual run. Correlation of the rate data with H_0 shows linearity with slope 1.25. More instructive is the fact that there is an induction period, a slight delay in the formation of *trans*-stilbene, indicating that an intermediate is formed during the course of the isomerization. The length of this induction period may be computed exactly from the rate of dehydration of 1,2-diphenylethanol under the same experimental conditions.

In 50% sulfuric acid, the dehydration of 1,2-diphenylethanol is 7.0 times faster than the isomerization of *cis*-stilbene; the product is exclusively *trans*-stilbene. Under similar conditions, racemization of optically active 1,2-diphenylethanol occurs some 58 times more rapidly than dehydration. The rate of dehydration of 1,2-diarylethanols is extremely sensitive to variation of substituents in the aromatic rings. As a case in point, the rate of dehydration of 1-*p*-anisyl-2-phenylethanol is 1070 times that of 1,2-diphenylethanol under comparable conditions. The rate data for 1-aryl-2-phenylethanols (five compounds) correlate with σ^+, $\rho = -3.77$, correlation coefficient 0.999. Examination of the fate of 1,2-diphenylethanol-2-d_1 reveals that the introduction of deuterium reduces the rate of dehydration by almost a factor of two, $k_H/k_D = 1.83$. However, the product composition is 78.7% *trans*-stilbene-α-d_1 and 21.3% *trans*-stilbene.

Thus, the rate-limiting process is the final elimination of a proton from the carbonium ion formed in small steady-state concentration, as depicted in eq. 1. These



(1)

observations on the mechanism of the acid-catalyzed dehydration of 1,2-diphenylethanol clearly imply that the rate-limiting step in the isomerization of *cis*-stilbene is the initial protonation of the olefinic system.[3] Confirmation of this view comes from a consideration of the solvent kinetic isotope effect.

When the isomerization of *cis*-stilbene is carried out in deuteriosulfuric acid (*cf.* Table I), the rate of isomerization is substantially reduced. The ratio k_{H_2O}/k_{D_2O} is 2.4 at 55% sulfuric acid; it increases to 3.0 at 65% sulfuric acid (comparisons made on a mole fraction basis).

Similar results were obtained with substituted stilbenes. Most striking is the solvent isotope effect in the case of *cis*-4,4′-dimethoxystilbene (*cf.* Table II).

TABLE II

SOLVENT ISOTOPE EFFECTS IN THE ACID-CATALYZED ISOMERIZATION OF SUBSTITUTED *cis*-STILBENES

cis-Stilbene	H_2SO_4,%	k_{H_2O}, sec.$^{-1}$	k_{D_2O}, sec.$^{-1}$[a]	k_{H_2O}/k_{D_2O}
—H	55 3	6.91×10^{-5}	2.88×10^{-5}	2.4
4-CH_3	55.3	5.50×10^{-4}	1.95×10^{-4}	2.8
4-OCH_3	55.3	1.23×10^{-2}[b]	2.95×10^{-3}	4.2
4,4′-$(OCH_3)_2$	55.3	4.68×10^{-2}[b]	7.76×10^{-3}[b]	6.0

[a] Interpolated to $N_{SO_4} = 0.1954$. [b] Extrapolated from data at lower acidities.

This variation in solvent isotope effect strongly suggests that the degree of carbon–hydrogen bond formation at the transition state is varying in a predictable manner with changes in the structure of the stilbene. As the stability of the resultant carbonium ion is increased, the extent to which carbon–hydrogen bond formation has been completed is lessened. Thus C–H

(1) D. S. Noyce and H. S. Avarbock, *J. Am. Chem. Soc.*, **84**, 1644 (1962).

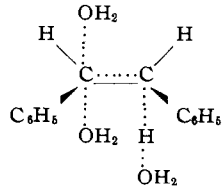
(2) D. S. Noyce, H. S. Avarbock, and W. L. Reed, *ibid.*, **84**, 1647 (1962).

(3) *Cf.* ref. 2, p. 1649.

bond formation is most nearly complete at the transition state in the reaction of *cis*-stilbene with acid; C–H bond formation is substantially less complete in the case of the reaction of *cis*-4,4′-dimethoxystilbene with acid. Further variation of isotope effects with structure is under active investigation.

Correlation of the isomerization rates for eleven mono- and disubstituted *cis*-stilbenes with substituent constants gives a reaction constant $\rho = -3.19$, correlation coefficient 0.995.

From these data and consideration of other solvolytic mechanism studies,[3] the transition state for the acid-catalyzed isomerization of *cis*-stilbene would appear to be best depicted by the following



It is clear that the suggested scheme of Gandini and Plesch[4] does not apply in aqueous media; it is unlikely that their suggested scheme will have any generality in polar solvents.

Acknowledgments.—Grateful acknowledgment is made to the donors of the Petroleum Research Fund for a grant in partial support of this work. Partial support was also provided by grants from the National Science Foundation (NSF-G-13125 and NSF-G-P-1572). We also wish to acknowledge helpful correspondence with Professor C. A. Kingsbury of Iowa State University and with Professor W. M. Schubert of the University of Washington.

(4) A. Gandini and P. H. Plesch, *Proc. Chem. Soc.*, 113 (1964).
(5) Shell Fellow in Chemistry, 1963–1964.
(6) National Science Foundation Cooperative Fellow, 1962–1964.

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RECEIVED JULY 2, 1964

The Comparison of Protein Structure in the Crystal and in Solution Using Tritium–Hydrogen Exchange[1]

Sir:

We have measured the exchange rate of tritium for the labile hydrogen atoms of insulin in the crystal and in solution; the data bear upon structural differences between the protein in the two phases.

Recent successes in determining the crystal structure of proteins[2,3] have greatly deepened our understanding of them, but since it is the molecule in solution which is generally of chemical and physiological interest, some assessment must be made of changes in structure possibly attending crystallization. In several recent reports[4–6] specific reactions of the crystalline protein have been used for comparisons made with this problem in mind. A method is needed, however, which can rapidly scan a number of crystal–solution systems and which reflects the over-all structure of the molecule rather than a single region of it. Tritium–hydrogen exchange is a technique suitable in both of the above respects.

Eli Lilly crystalline beef zinc insulin was dissolved at high pH and recrystallized at pH 6 to 7. The salt introduced in changing the pH was removed by washing five times with deionized water. To start the exchange, tritiated water was added to an aliquot of the crystal suspension after adjustment of the pH to 7.1. Solutions of insulin were prepared for reaction by dissolving crystals at pH 10 to 11, adjusting the pH to 7.1, and adding tritiated water. Because of the kinetics of crystal formation, both solution and crystal were stable at pH 7.1 for the duration of the experiment. The exchange was measured by the method of Leach and Hill.[7] For calculation of the hydrogen exchanged a molecular weight of 5733 was used, and protein concentrations were determined using an extinction coefficient, $E^{1\%}_{280\ m\mu}$ 8.65.

Table I shows the number of hydrogens exchanged in crystalline and dissolved insulin at pH 7.1 and 0°, at times from mixing to 2 days. Significant differences

TABLE I
TRITIUM–HYDROGEN EXCHANGE OF INSULIN IN THE CRYSTAL AND SOLUTION

Time, hr.	Crystals	Solution
0	50	61
5	61	75
26	64	82
49	66	84

between the crystal and solution were observed in the number of both instantaneously and nonexchanging hydrogens (11 and 18, respectively, of a total of approximately 84 hydrogens potentially exchangeable at this pH). These differences must be ascribed to[8]: (1) intermolecular interactions in the crystal which result in immobilization of labile hydrogens; (2) a greater flexibility of the structure in solution, permitting more rapid exchange of slowly exchanging hydrogens; or (3) a fundamental structural difference. An immobilization of 10 to 20 hydrogens by new intermolecular interactions in the crystal is unlikely in view of the few contacts of this type found in crystalline myoglobin (five to ten interactions involving polar groups,[2] in a protein three times larger than insulin). The differences are then likely to reflect an altered structure in the crystal, which may be either one of decreased flexibility (a different effective or time-average conformation) or one with basically different folding. Preliminary work has shown that the exchange behavior of insulin in an amorphous precipitate is like that of dissolved insulin, suggesting that the exchange differences between crystal and solution are the result of differences in folding, and not in flexibility or intermolecular interactions. Also of interest in this regard will be the results of exchange studies on myoglobin, which should be compatible with an unambiguous and detailed interpretation, since the hydrogens involved in

(1) This investigation was supported by research grants GM-9410, from the division of General Medical Sciences, Public Health Service, and G-20130, from the National Science Foundation.
(2) J. C. Kendrew, *Brookhaven Symp. Biol.*, **15**, 216 (1962).
(3) F. M. Richards, *Ann. Rev. Biochem.*, **32**, 269 (1963).
(4) M. S. Doscher, and F. M. Richards, *J. Biol. Chem.*, **238**, 2399 (1963).
(5) L. J. Banaszak, P. A. Andrews, J. W. Burgner, E. H. Eylar, and F. R. N. Gurd, *ibid.*, **238**, 3307 (1963).
(6) J. A. Rupley, *Biochemistry*, in press.
(7) S. J. Leach, and J. Hill, *ibid.*, **2**, 807 (1963).
(8) The diffusion rate of tritiated water into the crystal cannot affect the number of hydrogens exchanged at long times of reaction; moreover, it probably does not affect the number of instantaneously exchanged hydrogens, in view of the instantaneous and reversible titration of the ionizable groups of crystalline hemoglobin.[6]