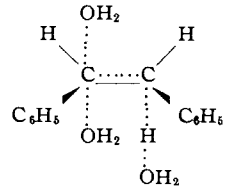
bond formation is most nearly complete at the transition state in the reaction of *cis*-stilbene with acid; C–H bond formation is substantially less complete in the case of the reaction of *cis*-4,4′-dimethoxystilbene with acid. Further variation of isotope effects with structure is under active investigation.

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.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720

D. S. NOYCE
D. R. HARTTER[5]
F. B. MILES[6]

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]

specific inter- and intramolecular interactions are known for crystals of this protein. Several other systems of crystallographic interest are being considered, in particular ribonuclease, lysozyme, and carboxypeptidase.

Acknowledgments.—We are indebted to Mr. John Stafford for his assistance in this work and to Dr. Raymond Hannapel for the use of a liquid scintillation counter.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

M. PRAISSMAN
J. A. RUPLEY

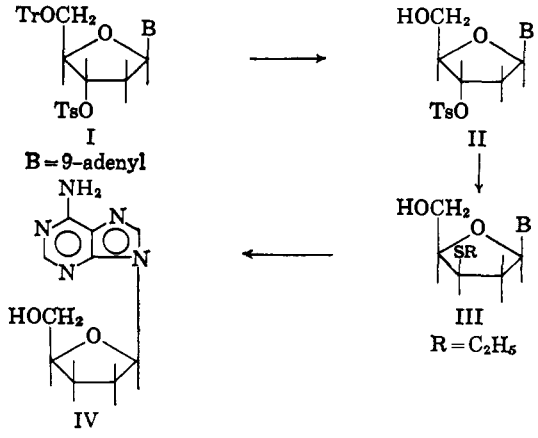
RECEIVED JUNE 29, 1964

The Synthesis of 2',3'-Dideoxyadenosine from 2'-Deoxyadenosine

Sir:

We wish to report the first preparation of the unusual purine nucleoside 2',3'-dideoxyadenosine (IV). The present synthesis utilizes the first recorded successful acidic removal of the 5'-triphenylmethyl (trityl) blocking group from a derivative of 2'-deoxyadenosine. The reaction scheme also employs the displacement of a secondary tosylate by alkyl mercaptide and emphasizes this reaction as a powerful new synthetic tool in the preparation of deoxynucleosides.

Interest in 2',3'-dideoxyadenosine (IV) arises from the fact that such a compound (as a 5'-phosphate derivative) should inhibit biosynthesis of DNA by acting as a polynucleotide chain terminator due to the



absence of the 3'-hydroxyl group. A related nucleoside antibiotic cordecypin[1-3] has recently been shown to be identical with 3'-deoxyadenosine.[4-6] The action of cordecypin on nucleic acid synthesis appears to be due to the accumulation of phosphorylated derivatives of the antibiotic[7,8] which are not able to substitute for structurally related adenosine phosphates due to the missing 3'-hydroxyl. There is also good evidence that such a pool of the purine 3'-deoxynucleotide acts as a specific inhibitor of purine nucleotide biosynthesis.[9,10] Although 3'-deoxyadenosine is an inhibitor of both DNA and RNA synthesis,[11,12] 2',3'-dideoxyadenosine would be expected to exert selective inhibition of DNA biosynthesis.

The synthesis and biological activity of 9-(tetrahydro-2-furyl)adenine[13,14] suggested some time ago that 2',3'-dideoxyadenosine would be a compound of considerable biochemical interest due to its closer structural relationship to 2'-deoxyadenosine. In the present study 5'-O-trityl-2'-deoxyadenosine[15] was treated with *p*-toluenesulfonyl chloride in pyridine to yield 5'-O-trityl-3'-O-tosyl-2'-deoxyadenosine (I) which was purified on alumina to give a chromatographically homogeneous foam in 65% yield. *Anal.* Calcd. for $C_{36}H_{33}N_5O_5S$: C, 66.8; H, 5.14; N, 10.8. Found: C, 66.8; H, 5.43; N, 10.7. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,500), $\lambda_{shoulder}^{MeOH}$ 226 mμ (ε 24,400); infrared band 705 (OTr) and 1170 cm.$^{-1}$ (OTs). Previous detritylations of purine 2'-deoxyribofuranoside derivatives have met with very limited success.[15-17]

Khorana and co-workers[18] have recently made use of the more acid labile tris(*p*-anisyl)methyl group in order to circumvent simultaneous cleavage of the purine base during deblocking. The study of this problem in our laboratory revealed that the 3'-tosyl function (I) contributed significantly to the stability of the glycosidic linkage.[19] Thus 5'-O-trityl-3'-O-tosyl-2'-deoxyadenosine (I) was heated for 12 min. at 100° in 80% acetic acid to give an essentially quantitative yield of 3'-O-tosyl-2'-deoxyadenosine (II) which was recrystallized from an ethanol–ether mixture to give fine needles, m.p. 184–184.5°. *Anal.* Calcd. for $C_{17}H_{19}N_5O_5S$: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.6; H, 4.59; N, 17.3. Spectral data showed: λ_{max}^{MeOH} 259 and 228 mμ (ε 15,900 and 13,500); strong infrared band at 1170 cm.$^{-1}$, band at 705 cm.$^{-1}$ absent. This would appear to provide a general synthetic route to a variety of previously inaccessible 3'-substituted derivatives of purine 2'-deoxynucleosides by replacement of the tosylate group. Nucleophilic displacement of the tosylate of 3'-O-tosyl-2'-deoxyadenosine (II, 6.5 g.) with ethyl mercaptide in a sodium ethoxide–ethanol solution at 80° yielded 1.2 g. (25%) of 6-amino-9-(3'-S-ethyl-3'-thio-2',3'-dideoxy-β-D-*threo*-pentofuranosyl)purine (III) by an assumed Walden inversion. III crystallized from ethanol in colorless needles, m.p. 210–212°. *Anal.* Calcd. for $C_{12}H_{17}N_5O_2S$: C, 48.8; H, 5.76; N, 23.7. Found: C, 48.7; H, 5.72; N, 23.6. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,600). Sponge nickel[20] de-

(1) K. G. Cunningham, S. A. Hutchinson, W. Manson, and F. S. Spring, *J. Chem. Soc.*, 2299 (1951).
(2) N. M. Kredich and A. J. Guarino, *Biochim. Biophys. Acta*, **41**, 363 (1960).
(3) N. M. Kredich and A. J. Guarino, *ibid.*, **47**, 529 (1961).
(4) W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1906 (1961).
(5) E. A. Kackzka, E. L. Dulaney, C. O. Gitterman, H. B. Woodruff, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 452 (1964).
(6) E. A. Kackzka, N. R. Trenner, B. Arison, R. W. Walker, and K. Folkers, *ibid.*, **14**, 456 (1964).
(7) H. Klenow, *ibid.*, **5**, 156 (1961).
(8) H. Klenow, *Acta Chem. Scand.*, **17**, 893 (1963).
(9) F. Rottman and A. J. Guarino, *Federation Proc.*, **22**, 2299 (1963).
(10) H. Klenow and K. Overgaard-Hansen, *Biochim. Biophys. Acta*, **80**, 500 (1964).
(11) H. Klenow, *ibid.*, **76**, 354 (1963).
(12) S. Frederiksen, *ibid.*, **76**, 366 (1963).
(13) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).
(14) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *J. Med. Chem.*, **6**, 471 (1963).
(15) W. Anderson, D. H. Hayes, A. M. Michelson, and A. R. Todd, *J. Chem. Soc.*, 1882 (1954).
(16) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958).
(17) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, p. 127.
(18) See H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3821 (1963), and references cited therein.
(19) D. M. Brown, G. D. Fasman, D. I. Magrath, and A. R. Todd, *J. Chem. Soc.*, 1448 (1954).
(20) Davison Sponge Nickel Catalyst, W. R. Grace & Co., Davison Chemical Division, Cincinnati, Ohio.