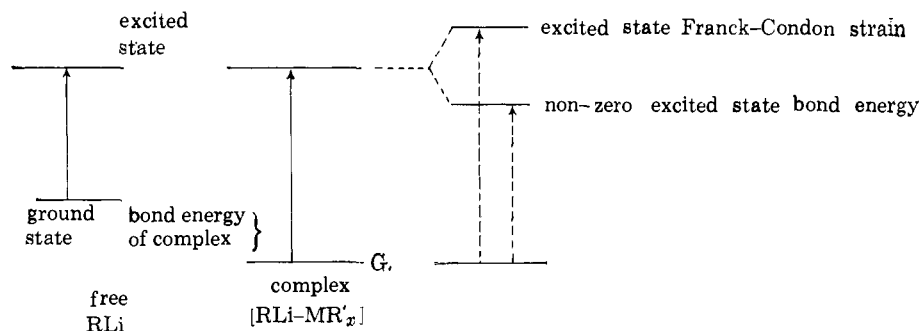


SCHEME I: GROUND STATE-EXCITED STATE RELATIONSHIP



4.29) in tetrahydrofuran.⁸ Diethylzinc added to I decreases the intensity of the 496 $m\mu$ absorption (eventually changing the color to orange) and produces a new absorption band at 375 $m\mu$.⁹ This is illustrated in Fig. 1. The molar absorptivity of the complex is 5.4×10^3 . Complete reversibility was demonstrated by first adding sufficient diethylzinc to reduce the absorption of I to $\sim 10\%$ of its original value, and then an excess of nonabsorbing organolithium compound (*n*-butyllithium¹⁰) to regenerate $\sim 95\%$ of the original absorption of I. That the recovery was not 100% is due to a slow ($\sim 5\%$ in 40 min.) time dependent decrease in the over-all absorptivity of the solution, which is probably due to solvent cleavage by the complex.² With aluminum alkyl complexes of I the decrease in absorptivity is much more rapid.

Equilibrium constants are calculated from the known amounts of added Lewis acid (the diethylzinc was used as a 1.5 *M* solution in hexane) and the accompanying decrease in absorptivity of I at 496 $m\mu$. Consistent values are obtained for the association constant between diethylzinc and I calculated as a 1:1 complex, *i.e.*, $[R^-Li^+] + Et_2Zn \rightleftharpoons [RZnEt_2^-Li^+]$. For eight data the average value of *K* is 103 l./mole with ± 1.4 average deviation from the mean. No time effects were observed, thus equilibrium must be established very rapidly.

The bond energy of the complex is obtained by the difference between the transition energy of the complex and that of the free organolithium compound. In 1,1-diphenyl-*n*-hexyllithium the carbon-lithium bond electrons may be considered to have partial p-character and are able to delocalize over the π -system. Owing to electron delocalization, I is an odd alternate ion in which the mobile electron pair of the C-Li bond occupies (to a first approximation) a nonbonding orbital. In the first electronic excited state, one of these electrons is promoted to the lowest antibonding π -orbital. Participation of the C-Li bond electrons in a dative bond lowers their energy.¹¹ In the electronic excited state the dative bond of the complex is presumably very weak (it now being a single electron bond), so the energy of the excited state is assumed to be unchanged by complex formation. This relationship is shown in Scheme I. Identifying the difference between the transition energy of the complex and the uncomplexed organolithium compound with the energy of the dative bond is analogous to the procedure used by Brealey and Kasha¹² in evaluating the strength of hydrogen bonds formed between alcohols and carbonyl compounds. In

(8) R. Waack and M. A. Doran, *J. Am. Chem. Soc.*, **85**, 1651 (1963).

(9) To illustrate this technique, we will detail our results for diethylzinc. A more complete report for other Lewis acids is in preparation.

(10) Diethylzinc in THF or butyllithium and diethylzinc in THF have only end absorption at short wave length.

(11) The positive lithium counter-ion may also be thought of as a vacant orbital acceptor. The transition energy of the completely ionized organolithium should, then, be less than when the carbon-lithium bond has more covalent character: R. Waack and M. A. Doran, *J. Phys. Chem.*, **67**, 148 (1963).

(12) G. J. Brealey and M. Kasha, *J. Am. Chem. Soc.*, **77**, 4462 (1955).

this manner a bond energy of 18.6 kcal. is obtained for the dative bond between diethylzinc and I. The bond energies so determined may not be exact, owing to two effects.¹³ If the bond energy of the excited state of the complex is not zero, the bond energies we obtain will be smaller than the true bond energy by the amount of the excited state bond energy. Secondly, equilibrium solvent orientation and distances in the ground state of the complex may not be the most favorable arrangement in the excited state. Because the solvent cannot reorient during the electronic transition, the observed difference in transition energy may be larger than the bond energy by the amount of the Franck-Condon strain. The transition of the free organolithium compound, however, should also be subject to similar excited state Franck-Condon strain. These effects are presumably small and of similar magnitude for a series of complexes.

We are currently using this spectroscopic technique to study the complexes formed between other Lewis acids and with other organolithium compounds. We have also examined the p.m.r. spectrum of these complexes which demonstrates conclusively that negative charge migration from the organolithium compound to the Lewis acid occurs on complex formation. This report is in preparation.

(13) G. C. Pimentel, *ibid.*, **79**, 3323 (1957).

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RECEIVED JULY 17, 1963

Insulin Peptides. IX. The Synthesis of the A-Chain of Insulin and its Combination with Natural B-Chain to Generate Insulin Activity

Sir:

We wish to report the synthesis of the A-chain of insulin and its combination with natural B-chain to generate insulin activity. This represents the partial synthesis of a protein possessing biological activity.

Degradative studies by Sanger and co-workers led to the elucidation of the amino acid sequence of insulin from various species and the postulation of a complete structure for this protein.¹ The proposed structure for sheep insulin is shown in Chart I.

Studies have been undertaken in our Laboratory directed toward the synthesis of this protein.^{2,3} The assumption was made that if chemically synthesized A- and B-chains are available the eventual synthesis of insulin can be achieved by combination of the two chains. This assumption was substantiated by the recent reports of Dixon and Wardlaw,⁴ of Wilson, *et al.*,⁵

(1) F. Sanger and H. Tuppy, *Biochem. J.*, **49**, 463, 481 (1951); F. Sanger and E. O. L. Thompson, *ibid.*, **53**, 353, 366 (1953); H. Brown, F. Sanger, and R. Kitai, *ibid.*, **60**, 556 (1955); J. I. Harris, F. Sanger, and M. A. Naughton, *Arch. Biochem. Biophys.*, **65**, 427 (1956).

(2) P. G. Katsoyannis, *J. Polymer Sci.*, **49**, 51 (1961).

(3) P. G. Katsoyannis, K. Fukuda, and A. Tometsko, *J. Am. Chem. Soc.*; **85**, 1681 (1963).

(4) G. H. Dixon and A. C. Wardlaw, *Nature*, **188**, 721 (1960).

preparation and gave a sharp, single Pauly-positive spot on paper chromatography ($R_f^{18} 0.4$, $R_f^{19} 0.5$) and a sharp, single Pauly-positive spot on high voltage paper electrophoresis.

Combination²⁰ of the synthetic material obtained by column chromatography with natural B-chain²¹ generated insulin activity in a yield of 0.5–1.2% (corrected for water content). This compares favorably with the activity obtained^{4,5,7} upon recombination of natural A- and B-chains. Insulin activity was determined by the mouse-diaphragm method²² and by immunological assays. In the latter case the insulin activity was neutralized by anti-ox-insulin serum.²³

Our studies toward the synthesis of the B-chain will be reported in a later communication.

(18) The R_f refers to an ascending chromatography in the system (see ref. 6) 2-butanol-acetic acid-8 M urea, 12.5:0.9:11.5.

(19) The R_f refers to a descending chromatography in the system (see ref. 6) 2-butanol-acetic acid-8 M urea, 12.5:1:11.5.

(20) We wish to express our gratitude to Dr. G. H. Dixon and Dr. S. Wilson of the Department of Biochemistry, and Connaught Medical Research Laboratories of the University of Toronto, Canada, for carrying out the combination experiments of the synthetic A-chain with the natural B-chain and the biological assays.

(21) Natural B-chain from ox insulin was used in the combination experiments.

(22) A. C. Wardlaw and P. J. Moloney, *Can. J. Biochem. Physiol.*, **39**, 695 (1961).

(23) P. J. Moloney and M. Coval, *Biochem. J.*, **59**, 179 (1955).

(24) This work was supported by a Research Career Development Award (GM-K3-15151) from the Public Health Service and a grant (A-3067) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, for which we wish to express our appreciation.

(25) The authors wish to express their appreciation to Mrs. Jemele Hudson for the enzymatic analyses and amino acid analyses and Miss Joanne Janos for technical assistance.

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RECEIVED AUGUST 12, 1963

Possible Vinyl Cation Intermediates, a 1,4-Chlorine Shift, and a Trimerization in the Reaction of Trifluoroacetic Acid with Alkynes¹

Sir:

Vinyl cations are reputed to be of relatively high energy as judged in part by the well known unreactivity of vinyl halides toward alcoholic silver nitrate.²

We wish to report that addition of trifluoroacetic acid to alkynes occurs almost as rapidly (approximately 0.2 as fast) as the previously studied comparable reaction of alkenes.^{3,4} Vinylic trifluoroacetates are formed which may be regarded as arising from intermediate substituted vinyl cations, as illustrated for the reaction of 1-hexyne.⁵

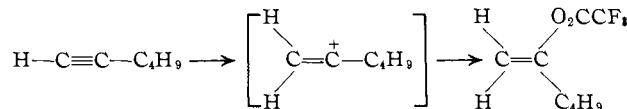
(1) This research was supported by a grant (790 A) from the Petroleum Research Fund of the American Chemical Society.

(2) (a) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, p. 148. Resonance stabilization of the vinyl halides presumably also contributes to the lack of reactivity of these compounds. (b) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th Ed., John Wiley and Sons, New York, N. Y., 1956, p. 141. (c) We have been unable to locate a reference to a definitive study of the reactivity of vinyl halides toward alcoholic silver nitrate. We suspect that the widespread familiarity of chemists with the statement in ref. 2b that "vinyl halides . . . are unreactive . . ." accounts for the "well known" aspect of the reaction.

(3) (a) P. E. Peterson and G. Allen, *J. Org. Chem.*, **27**, 1505 (1962); (b) P. E. Peterson and G. Allen, *ibid.*, **27**, 2290 (1962).

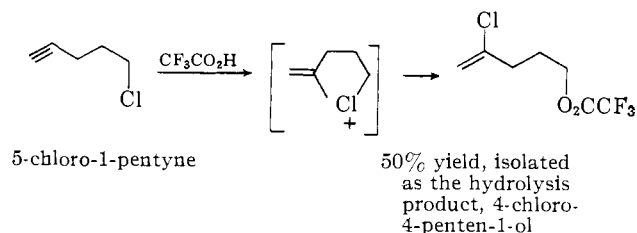
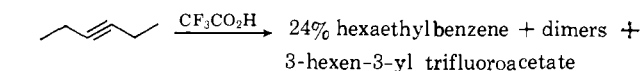
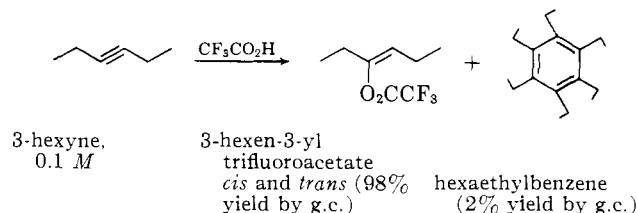
(4) The hydration of alkynes to ketones in the presence of strong acids has long been known, but either concentrated sulfuric acid or high temperatures have been employed. Cf. R. E. Schaad and V. N. Ipatieff, *J. Am. Chem. Soc.*, **62**, 178 (1940). Addition of hydrogen bromide to alkynes has been studied: M. S. Kharasch, J. G. McNab, and M. C. McNab, *ibid.*, **57**, 2463 (1935).

(5) We employ simple carbonium ion mechanisms in this communication in the absence of more detailed information regarding the nature of the intermediates or transition states involved. From the observed Markovnikov

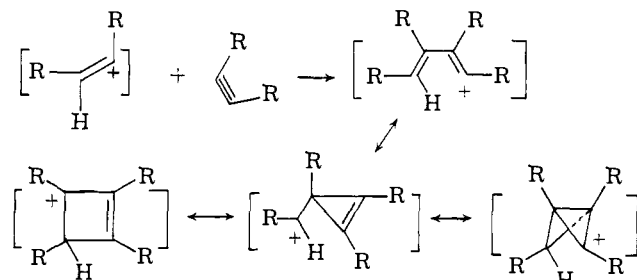


If vinyl cations are, in fact, intermediates several interesting questions arise. Will the cationic carbon atoms exhibit sp or sp² hybridization? Can the ready formation of these presumed energetic ions be accounted for by the high energy of the alkyne starting materials relative to the transition stage for reaction, as might be inferred from the large heats of hydrogenation of alkynes (to alkenes) relative to that of alkenes?⁶ Will unusual reaction products, possibly characteristic of hot carbonium ions, be formed?

Some initial observations concerning reaction products are illustrated by the following equations.



The formation of hexaethylbenzene is of interest because products of this type have previously been found most commonly in reactions catalyzed by metallic species and possibly involving cyclobutadiene complexes as intermediates in some instances.⁷ Although acid-catalyzed trimerizations of alkynes are not completely unknown,⁸ the ready formation of hexaethylbenzene in our reaction takes on added interest when some of the possibilities for classical and/or nonclassical resonance in the presumed intermediate dimeric ion are considered.



The reaction of 5-chloro-1-pentyne probably proceeds via the intermediate cyclic ion shown, which, interest-orientation and from preliminary studies of the effect of inductive substituents, however, the reaction of alkynes is similar to that of alkenes in regard to the positive character of the transition state.

(6) J. B. Conn, G. B. Kistiakowski, and E. A. Smith, *J. Am. Chem. Soc.*, **61**, 1868 (1939).

(7) (a) Cf. J. C. Sauer and T. L. Cairns, *ibid.*, **79**, 2659 (1957); (b) C. McKinley, *Ind. Eng. Chem.*, **44**, 995 (1952).

(8) See Almedigen, *Zh. Russ. Fiz. Khim. Obschestva*, **13**, 392 (1881); *Chem. Zentr.*, **12**, 629 (1881); cf. Beilstein's "Handbuch der Organischen Chemie," 4th Ed., Vol. 1, p. 249, for a report of the sulfuric acid-catalyzed trimerization of 2-butyne to hexamethylbenzene.