but at pH values below 7 its change is too small to be reported without full statistical analysis.

Optical rotation differences between CT and modified forms are greatest at low and at high pH values. Since b_0 differences become large at pH values above 8, it is probable that two different domains of the protein dominate our observations—one at low pH, the other at high. Above pH 8 reliable data for HC binding have not been obtained since HC greatly increases the autolysis rate of CT. In the pH region below 7.5 a_0 differences appear to consist of a large pH-independent change supplemented by a smaller pH-dependent variation which reflects the pH dependence of a_0 in unmodified CT. As measured by a_0 the normal conformation for catalysis appears to be that at pH 7.5 and the larger change mentioned above is either a consequence of acylation and substrate binding or a necessary preliminary process.

Acetyl-D-tyrosine ethyl ester (A-D-TEE) produces no change in rotation at pH 2.4. With ATEE rotation change shows a first-order dependence on substrate concentration with an equilibrium constant at pH 2.5 of approximately $2 \times 10^{-3} M$. Dialysis-equilibrium studies[2] show only one binding site. Hence rotation changes are to be attributed to the binding of one molecule of ATEE and not to any indirect effect of ATEE on solvent properties. Rotation changes due to HC at pH 4 are also first-order in HC concentration with an equilibrium constant of $6 \times 10^{-3} M$ in good agreement with constants from fluorescence studies,[9,10] from virtual substrate binding studies[2] and from some studies of HC as a competitive inhibitor of CT catalysis.[11,12] Hence, again we can attribute the effect to binding of substrate. In current interpretations, changes in a_0 without corresponding b_0 changes are due to changes in freedom of vicinal groups or environment composition at asymmetric carbons. Hence a_0 variations are to be attributed to conformation changes, though these may involve only alterations in structural rigidity and conformational relaxation times. There is no proof in this work that substrates influence conformational properties through binding to groups of the catalytic site. However, though a second binding site may be involved, there is thus far no evidence which makes this a probable situation. Substrate binding at low pH can be divided into two parts: (1) a change in conformational properties and (2) direct interaction of substrate and protein at the binding site. At pH 7.5 the change in conformation indicated by a_0 is a minimum and the direct interaction appears to dominate the thermodynamics. However, fluorescence studies to be reported suggest that this analysis is too simple.

The conformational changes may prove to be trivial or too sluggish to be related to fast catalytic processes.[10] On the other hand, the relationship between ΔH or ΔS for virtual substrate binding[2] and the pH dependence of a_0 for CT indicates an important relationship between the pH-dependent conformational processes associated with the formation of the specific nucleophilic site for catalysis and virtual substrate binding. There are relatively small but essential readjustments in the protein fabric which convert CT from a passive binding agent to a catalyst and these are directly or indirectly coupled to substrate binding. The effects of substrate binding are similar to and, where comparisons are possible, as large as those produced by specific acylation. The data suggest that if specific acylation occurs at the catalytic site, so does the binding of more

(9) S. Yanari, R. Lumry and F. Bovey, to be published.

(10) J. M. Sturtevant, *Biochem. Biophys. Res. Commun.*, **8**, 321 (1962).

(11) H. Neurath, J. A. Gladner and G. DeMaria, *J. Biol. Chem.*, **188**, 407 (1951).

(12) R. J. Foster and C. Niemann, *J. Am. Chem. Soc.*, **77**, 3370 (1955).

normal substrates responsible for the changes in a_0. It is important to note, however, that there is no basis in this work upon which to postulate the occurrence of important conformation changes during normal catalysis. Of the substances tested only diisopropylfluorophosphate produces significant a_0 changes at pH 7.5 and there is no reason to suppose that the interaction of this substance with CT parallels the processes of normal catalysis. Current high-speed kinetic studies and static fluorescence studies may provide some basis for postulating conformational changes during normal catalysis and these will be reported. Several authors have proposed that primary bond rearrangement within the protein is essential to the formation of the specific nucleophilic site.[13] It is our thesis that an equally essential aspect of the development of this site is the distortion of primary bonds which provides the necessary local electronic properties for catalysis.[14] We shall defend this thesis and present details of our results in forthcoming papers.

Experimental.—Rotatory dispersion measurements were made with a Rudolph Model 200 spectropolarimeter modified to increase ease of reading and precision. Protein concentrations were usually 0.3%. The molecular weight of the protein was taken as 24,800. The routine standard deviation in a_0 is 3° but variations in protein concentration often increase this error to 5°. The λ_0 parameter was 2390 Å., the best-fit value found by Brandts.[5] Worthington CT and chymotrypsinogen were used throughout without further purification. TMACT, acetylCT and DIPCT were made by well-known procedures.[15]

(13) S. Bernhard, 142nd National Meeting, American Chemical Society, Atlantic City, N. J., September, 1962, Abstract 26-O; H. N. Rydon, *Nature*, **182**, 928 (1958); J. A. Cohen, R. A. Oosterbaan, H. S. Jansz and F. Berends, *J. Cell Comp. Physiol.*, **54**, Suppl. 1, 231 (1959).

(14) R. Lumry and H. Eyring, *J. Phys. Chem.*, **58**, 110 (1954).

(15) A. K. Balls and F. L. Aldrich, *Proc. Natl. Acad. Sci. U. S.*, **41**, 190 (1955); A. K. Balls and H. N. Wood, *J. Biol. Chem.*, **219**, 245 (1956).

DEPARTMENT OF CHEMISTRY   HELEN PARKER
UNIVERSITY OF MINNESOTA   RUFUS LUMRY
MINNEAPOLIS 14, MINNESOTA

H ATOM ADDUCTS—NEW FREE RADICALS?

Sir:

The question as to whether molecules such as water or ammonia in the gas phase have an affinity for a hydrogen atom is of considerable interest since it implies the stability of free radicals of the type HA where A is a saturated proton acceptor molecule. An answer to this question may be obtained by considering the bond dissociation energies involved in a conventional hydrogen bonded system such as X–H. . .A. The bond dissociation energy in the isolated hydrogen donor molecule XH in the gas phase is given by

$$D(X - H) = \Delta H_f(X) + \Delta H_f(H) - \Delta H_f(XH) \quad (1)$$

where ΔH_f is the heat of formation in the gas phase.

In the complex

$$D(X-HA) = \Delta H_f(X) + \Delta H_f(HA) - \Delta H_f(XHA) \quad (2)$$

the enthalpy of hydrogen bond formation may be written as

$$-\Delta H = D(XH-A) = \Delta H_f(A) + \Delta H_f(XH) - \Delta H_f(XHA) \quad (3)$$

To construct a cycle we may write the bond dissociation energy of the HA radical as

$$D(H-A) = \Delta H_f(H) + \Delta H_f(A) - \Delta H_f(HA) \quad (4)$$

so that from these equations one readily obtains the

relation

$$D(\text{H-A}) = D(\text{X-H}) - D(\text{X-HA}) - \Delta H \qquad (5)$$

Since $-\Delta H$ is positive, $D(\text{H-A})$ is greater than zero if $D(\text{X-H}) - D(\text{X-HA})$ is either positive or negative but less than $-\Delta H$. An approximation of $D(\text{X-H})$ and $D(\text{X-HA})$ may be obtained from the Morse potential for XH and X-HA treated as diatoms and the vibrational frequencies $\nu(\text{X-H})$ and $\nu(\text{X-HA})$. For OH bonds for example it turns out that $D(\text{X-H}) - D(\text{X-HA})$ is about 5 to 25 kcal./mole in agreement with the expectation that the XH bond in the complex is longer and weaker than XH in the free molecule. Experimental values for $-\Delta H$ probably are in the range of 2 to 8 kcal./mole (if the solution values can be taken as a guide) so that the bond dissociation energy of the free radical HA is about 7 to 33 kcal./mole in the gas phase.

Perhaps low temperature hydrogen atom reactions in the gas phase or e.s.r. experiments on H atoms trapped in an inert matrix containing a diluted proton acceptor could reveal the presence of such radicals.

If the Morse function is a valid description for $D(\text{X-H})$ and $D(\text{X-HA})$, equation 5 gives a relationship between ΔH and the difference in stretching frequencies $\nu(\text{X-H}) - \nu(\text{X-HA})$. This is being investigated in more detail both experimentally and theoretically.

Helpful discussions with Drs. S. Bauer, K. Kutschke and F. P. Lossing are gratefully acknowledged.

DIVISION OF PURE CHEMISTRY
NATIONAL RESEARCH COUNCIL   H. J. BERNSTEIN
OTTAWA, CANADA

COMPLEX FORMATION BETWEEN ETHYLLITHIUM AND t-BUTYLLITHIUM

Sir:

Cosolution of ethyllithium and t-butyllithium in benzene leads to the formation of complex organolithium compositions, differing from either pure component, containing both types of alkyl groups bonded to lithium. Pure ethyllithium[1,2] and t-butyllithium[3] exist as six-fold and four-fold polymers, respectively, in benzene solution. Apparently carbon–lithium bond breaking takes place in this solvent, leading to exchange of alkyl groups between polymeric organolithium molecules when both compounds are present. The new compounds which are produced are believed to be electron-deficient polymers of the type $(\text{EtLi})_n(t\text{-BuLi})_{m-n}$, where m is a small number such as 4 or 6.

Ethyllithium alone is sparingly soluble in cold benzene, but its solubility is greatly enhanced in benzene solutions of t-butyllithium. Evaporation of the benzene from a solution of both compounds leaves a low-melting white solid residue. The white solid is highly soluble in pentane, unlike ethyllithium which is virtually insoluble in this solvent. The complex is also much more volatile than ethyllithium. It distils readily at 70° and 0.1 mm., forming colorless crystals. Gas chromatographic analysis of the hydrocarbons formed by hydrolysis of several samples of the distilled material show that its composition can vary. The ratio of ethyl to t-butyl groups in the resublimed composition is nearly the same as that in the original solution. Benzene solution with ethyllithium:t-butyllithium ratios of 1:1 and 1.8:1 gave distilled products with ethyl:t-butyl ratios of 1.1:1 and 1.7:1, respectively; m.p. 68–72° and 56–59°.

(1) T. L. Brown and M. T. Rogers, *J. Am. Chem. Soc.*, **79**, 1859 (1957).
(2) R. West and W. Glaze, *ibid.*, **83**, 3580 (1961).
(3) M. Weiner, G. Vogel and R. West, *Inorg. Chem.*, **1**, 654 (1962).

The infrared spectrum in the CsBr region of a benzene solution containing a 1:1 mixture of ethyllithium-7 and t-butyllithium-7 exhibits a strong broad band centered around 500 cm.$^{-1}$, not found in the spectra of either of the components. Ethyllithium and t-butyllithium have strong absorption bands at 538 and 480 cm.$^{-1}$, respectively, attributed to vibrations of the carbon–lithium framework. Summation of optical densities of spectra of ethyl- and t-butyllithium-7 show that the 500 cm.$^{-1}$ band does not result from overlapping of the bands of these components. The 500 cm.$^{-1}$ band in the EtLi^7–t-BuLi^7 complex shifts to about 514 cm.$^{-1}$ in a 1:1 solution of EtLi^6 and t-BuLi.^{7} This band may be tentatively assigned to carbon–lithium framework vibrations in one or more new organolithium species containing both ethyl and t-butyl groups.

The characteristic triplet and quartet pattern in the hydrogen n.m.r. spectrum of ethyllithium[4] in benzene is not changed by addition of t-butyllithium, and the peak positions are only slightly shifted. This finding is not inconsistent with complex formation, but does suggest that, if ethyl groups exist in different environments in the solution, exchange must be rapid compared to the relaxation time. On the other hand, the singlet at $\tau = 9.01$ in the proton n.m.r. spectrum of pure t-butyllithium in benzene is split into a doublet in solution containing ethyllithium. A 1:1 mixture of the two compounds gives t-butyl peaks at $\tau = 9.08$ and $\tau = 9.31$, with relative intensity about 7:2. Thus t-butyl groups appear to be present in at least two environments.
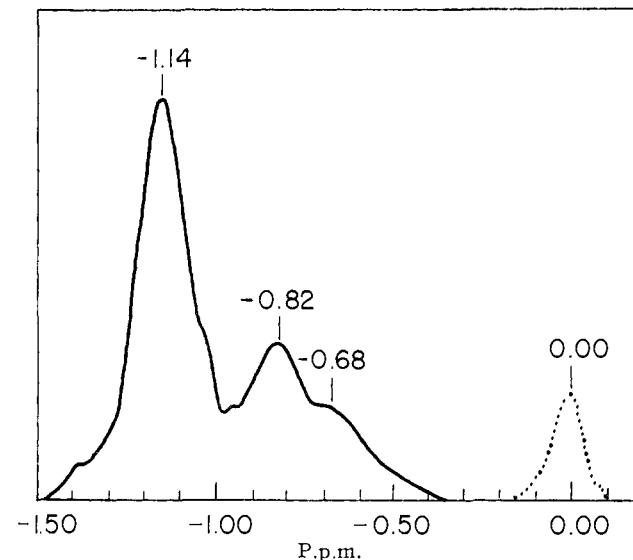

Fig. 1.—Lithium-7 n.m.r. spectra: solid line, 1:1 mixture of ethyllithium and t-butyllithium, each 8% by weight in benzene; dotted line, aqueous lithium nitrate solution as external reference.

Indication of the chemical interaction of ethyllithium and t-butyllithium in solution is also obtained from the lithium-7 n.m.r. spectrum. Pure ethyllithium and t-butyllithium in benzene give only single lithium resonances at -1.29 and -0.64 p.p.m., respectively, referred to aqueous lithium nitrate solution. A 1:1 mixture of the two compounds in the same solvent gives a spectrum consisting of three bands at -1.14, -0.82 and -0.68 p.p.m., with relative intensities about 5:2:1. In the mixture, lithium atoms must be present in at least three different environments, between which exchange is slow compared to the relaxation time.

(4) T. L. Brown, D. W. Dickerhoof and D. A. Bafus, *J. Am. Chem. Soc.*, **84**, 1371 (1962).