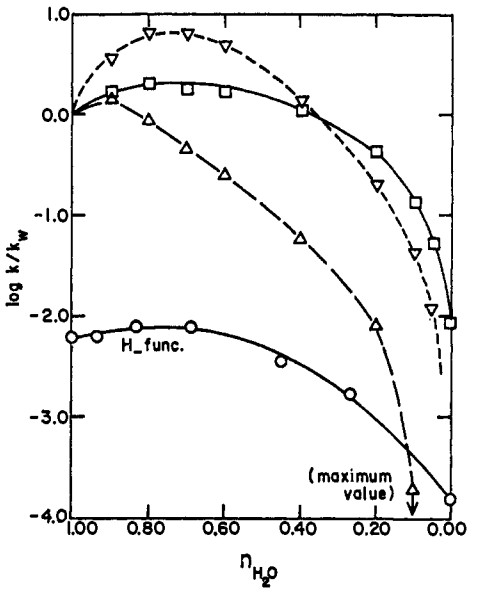


Figure 1. Dependence of relative rates of detritiation of *t*-butylmalononitrile-1-*t* on mole fraction of water for three solvent systems: water–dimethyl sulfoxide, ▽; water–ethanol, □; water–dioxane, △. The acidity function H_- for the water–ethanol system is plotted for comparison on a displaced scale.

It is tempting to interpret the initial rate acceleration which occurs upon addition of the nonaqueous component as due to the effect of these species on the structure of water. In terms of an explicit two-component interpretation of the structure of water, *i.e.*, "free" and bound water, of the kind discussed recently, for example, by Walrafen[7] and by Franks,[7] one could discuss the observed enhanced basicity qualitatively by postulating that the effect of the nonaqueous addition was initially to increase the amount of unbound water, with the further assumption that it is this component which is involved in the reaction with the tritiated acid species. However, other less specific considerations of structure change could also explain the rate acceleration. Since a number of thermodynamic and other properties of mixed water–nonaqueous solvent systems are known to go through maxima,[8,9] perhaps the kinetic maxima observed here are not surprising. The results do, however, demonstrate the existence of a probe of a somewhat different sort for studying these systems and suggest some significant further experiments.

Bates and Schwarzenbach[10] have studied the equilibrium acid–base properties of one of these solvent mixtures, water–ethanol, by several techniques. The set of systems of interest for comparison with the present studies contained 0.002 *M* HCl and 0.008 *M* NaCl; *i.e.*, the hydrogen ion concentration was held constant at a value close to that used here. Four different acidity functions were measured: two indicator acidity functions, H_0 and H_- and two electrometric functions, pH (conv) and pW$^{\oplus}$. Of these four, only one function, H_-, reasonably parallels the present kinetic measurements of basicity. This function is plotted in Figure 1 on a displaced scale for comparison, and it is evident that there is approximate parallelism with the kinetic data in the same solvent system.

Using BH and B^- for the two forms of the H_- indicator, 2,6-dinitro-4-chlorophenol, and using RCH and M* for the malononitrile and the transition state for hydrogen ion removal, the value of the activity coefficient ratio, $f_{BH}f_{M^*}/f_{RCH}f_{B^-}f_{H^+}$, is seen to be approximately independent of solvent composition. This is perhaps plausible in the high ethanol region since B^- and H^+ are probably extensively present as ion pairs and M* is itself essentially an ion pair of the type $RC^-\cdots H^+$. The rough parallelism between rate and acidity function in the high water region is, however, rather more surprising. Further solvent systems are under study.

(11) To whom requests for reprints should be sent.

F. Hibbert, F. A. Long,[11] E. A. Walters
Department of Chemistry, Cornell University
Ithaca, New York 14850
Received February 1, 1969

The Contribution of Circular Dichroism below 185 mμ to the Optical Rotatory Dispersion of Cytochrome b_2

Sir:

The optical rotatory dispersion (ORD) function of a substance, expressed as the molar rotation, $[M]_\lambda$, can be calculated[1] by means of the Kronig–Kramers relation (eq 1) from the circular dichroism (CD) function, in

$$[M]_\lambda = \frac{2}{\pi}\int_0^\infty [\Theta]_{\lambda'}\frac{\lambda'}{\lambda^2 - \lambda'^2}\,d\lambda' \qquad (1)$$

the form of the molar ellipticity, $[\Theta]_\lambda$. If the CD spectrum obtained in the observable range of wavelength, λ, extending down to a lower limit of approximately 185 mμ, gives on integration an ORD spectrum differing significantly from the observed spectrum, it is to be concluded that one or more bands of appreciable rotational strength lie outside the observable spectral region.

Schechter and Saludjian[2] reported that the ORD function for ferricytochrome *c* calculated from its CD spectrum lies below the observed function and concluded that the missing bands situated below 190 mμ are due to heme transitions. We have made similar observations[3,4] on another hemoprotein, cytochrome b_2. This enzyme contains one flavin mononucleotide and one protoheme group per 80,000 molecular weight. As shown by the data in Figure 1, obtained with a sample of enzyme having an activity[5] of 200 sec^{-1}, this protein exhibits a profusion of Cotton effects and CD extrema in the visible and ultraviolet regions of the spectrum. Both the ORD and CD data below 260 mμ, when interpreted in the usual manner,[6] indicate an apparent α-helical content of 17%.

(1) A. Moscowitz in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1960.
(2) E. Schechter and P. Saludjian, *Compt. Rend.*, **264**, 1501 (1967).
(3) J. M. Sturtevant and T. Y. Tsong, *J. Biol. Chem.*, **243**, 2359 (1968).
(4) J. M. Sturtevant and T. Y. Tsong, manuscript in preparation.
(5) R. K. Morton and J. M. Sturtevant, *J. Biol. Chem.*, **239**, 1614 (1964).
(6) (a) J. T. Yang in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., 1967; (b) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

(7) See, for example, review papers by G. E. Walrafen (p 9) and F. Franks (p 31) in "Hydrogen-Bonded Solvent Systems," A. K. Covington and P. Jones Ed., Taylor and Francis, Ltd., London, England, 1968.
(8) F. Franks and D. J. G. Ives, *Quart. Rev.* (London), **20**, 1 (1966).
(9) J. B. Hyne in ref 7, p 99.
(10) R. G. Bates and G. Schwarzenbach, *Helv. Chim. Acta*, **38**, 699 (1955).

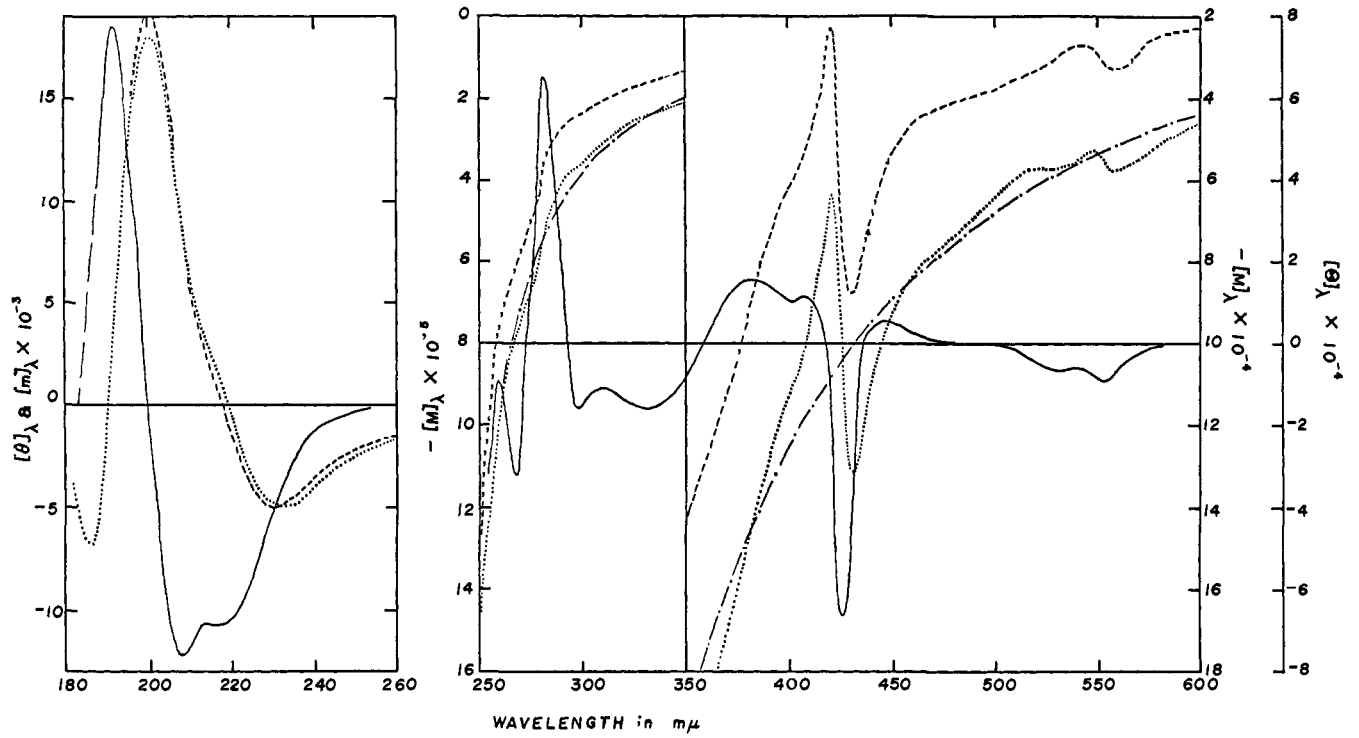


Figure 1. ORD and CD spectra of reduced cytochrome b_2. The quantities plotted are $[M]$ = molar rotation = $[\alpha]M/100$, where M = 80,000; $[m]$ = mean residue rotation = $[M]/610$ (610 amino acid residues per molecule); $[\Theta]$ = molar ellipticity = $\theta M/100$; $[\theta]$ = mean residue ellipticity = $[\Theta]/610$. ————, CD spectrum; - - - -, observed ORD spectrum; ···, ORD spectrum calculated from entire CD spectrum; —·—, ORD spectrum calculated from CD spectrum for $\lambda < 260$ mμ. Integration interval, 2 mμ; 10 μM enzyme in 5 mM L-lactate–0.05 M phosphate buffer, pH 7.0, 25°. Appropriate base lines with enzyme omitted have been subtracted.

Figure 1 includes two calculated ORD curves. One of these was calculated from the complete observed CD spectrum extended by extrapolation below 187 mμ as indicated in the figure, and the other from the CD spectrum observed below 260 mμ. In Figure 2, line A, the reciprocal of the difference in molar rotation between the observed and calculated ORD curves, is plotted against the square of the wavelength in the range 250–600 mμ. The linearity of this plot shows that the difference data fit a one-term Drude equation and suggests that there is a positive CD band centered at 150 mμ which makes an important contribution to the ORD of cytochrome b_2. Line B in Figure 2 shows a similar plot for a sample of enzyme estimated to be 26% helical. The Drude plot is not sufficiently sensitive to exclude the possibility that a complex of bands in the region of 150 mμ is involved.

Since the observed CD spectrum below 250 mμ is completely independent[4] of the oxidation state of the heme group and of the presence or absence of the FMN group, and since the apparent rotational strength (see below) of the band at 150 mμ parallels that of the band at 190 mμ, we believe that this new band cannot be assigned to a transition in either of the prosthetic groups. Several authors[7-9] have predicted an optically active band in this spectral region due to a transition in peptide groups in α-helical array.

It is interesting that the slopes of the plots in Figure 2 lead to estimates of rotational strengths which are consistent with assignment of the 150-mμ band to an α-helical peptide transition. The slope of line A,

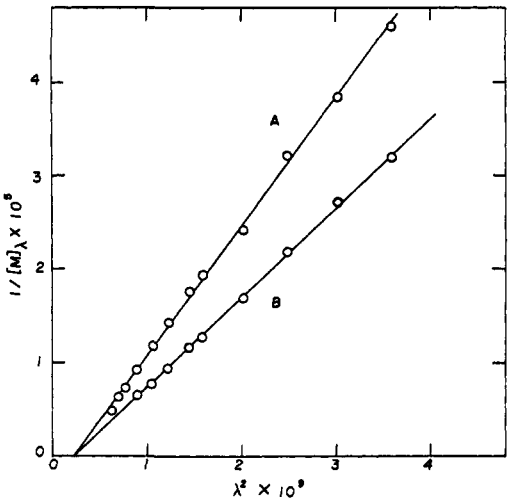


Figure 2. Plot according to a single-term Drude equation of the difference between the observed and calculated ORD data: (A, sample of enzyme used for data in Figure 1; B, sample of enzyme with 26% apparent α-helical content.

Figure 2, after conversion to mean residue rotations, gives[1,10] a value of $+5.6 \times 10^{-40}$ erg cm^3 for the rotational strength of the band at 150 mμ. This figure is increased to $+33 \times 10^{-40}$ erg cm^3 if the band is assumed to be due to α-helical peptide groups and allowance is made for the helical content of the protein. Holzwarth and Doty[6b] reported a value of $+81 \times 10^{-40}$ erg cm^3 for the rotational strength of the CD band at

(7) W. Moffitt, *Proc. Natl. Acad. Sci. U. S.*, **42**, 736 (1956).
(8) J. A. Schellman and P. Oriel, *J. Chem. Phys.*, **37**, 2114 (1962).
(9) R. W. Woody and I. Tinoco, Jr., *ibid.*, **46**, 4927 (1967).
(10) L. Rosenfeld, *Z. Physik*, **52**, 161 (1928).

190 mμ. The slope of line B in Figure 2 in similar manner gives the value $+34 \times 10^{-40}$.

Moffitt[11] plots derived from the (smoothed) observed ORD data in Figure 1, and from the ORD curve calculated from the CD data observed below 260 mμ, lead to $b_0 = -100°$, corresponding to 16% α helix, in agreement with the α-helical content inferred from the original ORD and CD spectra.

The results reported here show that the observed ORD spectrum of cytochrome b_2 can be quantitatively accounted for on the basis of the observed CD spectrum plus an additional positive CD band centered at 150 mμ. A possible interpretation of the data attributes this band to a transition involving peptide units in α-helical array and assigns to the band a mean residue rotational strength of $+33 \times 10^{-40}$ erg cm³.

Acknowledgment. Mr. S. Rudolph wrote the computer program for the integration of eq 1. The work was supported in part by Research Grant GM 04725 and Equipment Grant GM 12246 from the National Institutes of Health, U. S. Public Health Service. The authors are indebted to Professor John A. Schellman for very helpful suggestions.

(11) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, 42, 596 (1956).

Tian Yow Tsong, Julian M. Sturtevant
Department of Chemistry, Yale University
New Haven, Connecticut 06520
Received February 24, 1969

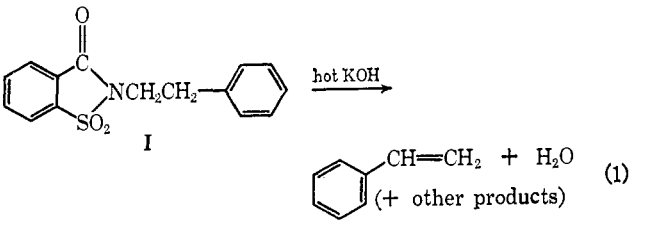
A New Approach to Deamination. III. A Simple Procedure for the High-Yield Conversion of Primary Aliphatic Amines into Alkyl Halides and Alkenes *via* the Use of Sulfonimide Leaving Groups

Sir:

One of the most frequently used activating groups for the E2 and SN2 cleavage of carbon–oxygen bonds in aliphatic alcohols is the *p*-toluenesulfonyl (*i.e.*, tosyl) group. Tosylate and related anions are presumably much better leaving groups than the hydroxide anion, mainly because of the relatively weakly basic nature of the tosylate anion.

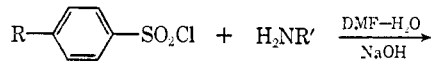
Analogous reasoning in respect to primary aliphatic amines leads to the prediction that sulfonimides should behave in a similar manner. In this event, a simple two- or three-step procedure for the conversion of primary aliphatic amines into alkyl halides and other alkyl derivatives would be available to the synthetic chemist for the first time.[1] Our previous work was concerned primarily with the use of the saccharin functionality as an activating group. We found that deamination only occurred with the saccharin derivatives of certain special amines such as 2-phenylethylamine (I) (eq 1). When simple alkylsaccharins were treated with nucleophiles such as iodide ion, aniline, or hot KOH, either no reaction was observed or the products of saponification were isolated.[2,3]

The search for a generally potent activating group for primary aliphatic amines, nonetheless, continued,

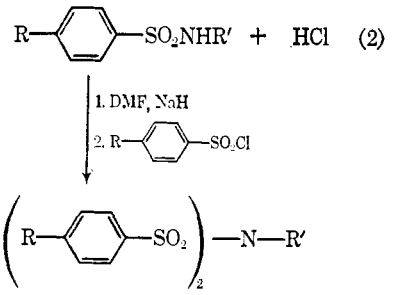


I (1)

and we wish to report that activating groups such as the N,N-di-*p*-toluenesulfonyl and the N,N-di-*p*-nitrobenzenesulfonyl functionalities (both are obtained in up to 95% over-all yield by twice treating the parent amine with the appropriate sulfonyl chloride; eq 2) are effective for promoting carbon–nitrogen bond cleavages of primary aliphatic amines. For example, we have found that activated amines such as N-*n*-hexyl-*p*-toluenesulfonimide (IIa) (mp 114–115°) and N-*n*-hexyl-*p*-nitrobenzenesulfonimide (IIb) (mp 132–133°) give, when treated with iodide ion in DMF at

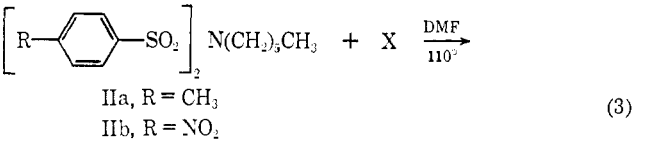


R = CH_3, NO_2
R′ = hexyl, cyclohexyl

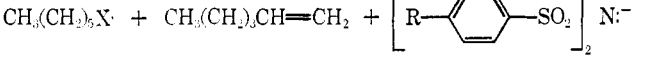


(2)

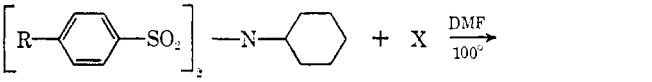
90–120° for 2–40 hr, *n*-hexyl iodide (up to 79% isolated yield), imide leaving group (up to 75%), and 1-hexene (0 to 31% yield) (eq 3). Similarly, N-cyclohexyl-*p*-toluenesulfonimide (IIIa) (mp 159–161°) and N-cyclohexyl-*p*-nitrobenzenesulfonimide (IIIb) (mp 192–193°) give, when treated with iodide ion in DMF at 90–150° for 40–69 hr, cyclohexene (up to 90% yield) and imide leaving group (up to 94% yield) (eq 4). Nucleophiles such as bromide anion and aniline likewise have been observed to displace the imide anion. Quantitative



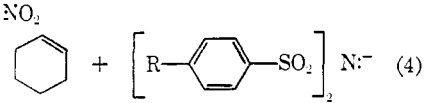
IIa, R = CH_3
IIb, R = NO_2 (3)



X = I^-, Br^-, $C_6H_5NH_2$



IIIa, R = CH_3
IIIb, R = NO_2



(4)

X = I^-, $C_6H_5NH_2$

(1) R. J. Baumgarten, *J. Chem. Educ.*, 43, 398 (1966).
(2) R. J. Baumgarten and P. J. DeChristopher, *Tetrahedron Letters*, 3027 (1967).
(3) R. J. Baumgarten, *J. Org. Chem.*, 33, 234 (1968).