lites: 11-hydroxyacronine (**a**) and 9-hydroxyacronine (**b**).

## Discussion

Although acronine contains both an *O*-Me and an *N*-Me group, dealkylation does not appear to be an important route of biotransformation. An exception to this observation occurs in the guinea pig. In this species and to a lesser extent in mice, O-demethylation does account for a portion of the metabolic conversion of acronine. In general, however, hydroxylation appears to be the major route of oxidation. Of the 5 species studied, all hydroxylate acronine at C-9 and C-11, indicating a similarity of the enzyme systems involved in each animal. The major species difference observed involved the ability to hydroxylate the methyl group at the quaternary C. This route was observed in rat, mouse, dog, and man, but not in guinea pig.

The observation that the alkaloid acronine is readily oxygenated by mammalian enzymes at a number of different positions in the molecule is of some interest since none of these metabolites have been reported as metabolites of acronine in *Acronychia bauri*. It would be of some interest to reinvestigate the alkaloids of this plant to see if any of the hydroxyacronines occur naturally.

The work described emphasizes the value of contemporary chromatographic methods used in conjunction with mass spectroscopy for the determination of the structure of natural products.

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# Choline Acetyltransferase Inhibitors. Physicochemical Properties in Relation to Inhibitory Activity of Styrylpyridine Analogs

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Hückel molecular orbital and Hansch calculations were performed on various styrylpyridine derivatives and analogs, some of which are potent inhibitors of choline acetyltransferase (ChA). The results are consistent with the view that these compounds bind to ChA *via* hydrophobic and $\pi$ donor contributions of the aryl moiety, and $\pi$ acceptor interactions, presumably by the pyridinium-like portion.

Previous reports[1,2] have described some derivatives and analogs of styrylpyridine which include quite potent inhibitors of choline acetyltransferase (EC 2.3.1.6)(ChA). These papers delineated, in a qualitative manner, steric and electronic features contributing to optimal inhibitory activity. Some speculations were made concerning the nature of possible interactions of these inhibitors with the enzyme at a molecular level. The present report describes some efforts to quantitate certain structure–activity relationships (SAR) among these types of compounds. Such exercises could serve to more critically evaluate proposed[2] inhibitor–enzyme interactions and also provide additional guidance to directions of future molecular design.

The application of computer based techniques for quantitative assessment of some S variables in SAR for various classes of medicinals has met with moderate success.[3] Of these methods, Hückel molecular orbital calculations[4] and the Hansch technique[5,6] have now been applied to the interpretation of the structural variables among styrylpyridine-like compounds with ChA inhibitory activity.

## Results and Discussion

Hückel molecular orbital (HMO) calculations were performed on 21 styrylpyridine derivatives and analogs. The method and parameters utilized are discussed in the Experimental Section. Compounds were divided into three structural groups (A, B, and C) for initial ease of comparison. These groups are presented in Table I together with the *in vitro* activity data[2] (expressed for convenience as log $1/I_{50}$) and various pertinent HMO derived quantities.

It has been suggested[2] that these compounds may bind to ChA *via* a charge transfer acceptor contribution by the pyridinium-like moiety and charge transfer donor interactions involving the aryl group. Using this as a working hypothesis, the data generated from the HMO calculations were examined for possible correlations. Disappointing correlations were obtained using either $E_{HOMO}$ and/or $E_{LEMO}$, the energies of the highest occupied and lowest empty molecular orbitals, respectively. These quantities are related to the ability of the molecule as a *whole* to act as a charge transfer donor or acceptor. This is not incompatible with the working hypothesis since inhibitor activity appears to require a separation of donor and acceptor units as in styrylpyridine; ring fusion of components as in phenanthridinium[1] results in loss of activity.

(1) C. J. Cavallito, H. S. Yun, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **12**, 134 (1969).

(2) C. J. Cavallito, H. S. Yun, T. Kaplan, J. C. Smith, and F. F. Foldes, *ibid.*, **13**, 221 (1970).

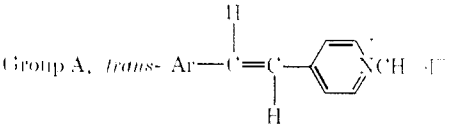
(3) See for example, (a) J. G. Beasley and W. P. Purcell, *Biochem. Biophys. Acta*, **178**, 175 (1969); (b) R. W. Fuller, M. M. Marsh, and J. Mills, *J. Med. Chem.*, **11**, 397 (1968).

(4) A. Streitweiser, "Molecular Orbital Theory for Organic Chemists," Wiley, New York, N. Y., 1961.

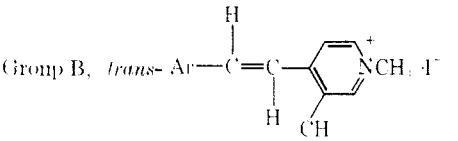
(5) C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, *J. Amer. Chem. Soc.*, **85**, 2817 (1963).

(6) T. Fujita, J. Iwasa, and C. Hansch, *ibid.*, **86**, 5175 (1964).

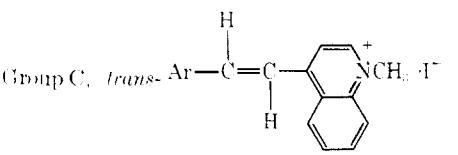
TABLE I
OUTPUT FROM HMO CALCULATIONS AND CORRELATIONS



| Ar | Log 1/$I_{50}$[a] | Log 1/$I_{50}$ calcd by eq 1 | Log 1/$I_{50}$ calcd by eq 11 | $\Sigma S_{Ar}^{E}$[b] | $\Sigma\|Q\|_{Ar}$[c] | $E_{LEMO}$[d] |
|---|---|---|---|---|---|---|
| 1-Naphthyl | 6.33 | 6.42 | 6.42 | 8.791 | 0.105 | −0.228 |
| 3-$BrC_6H_4$ | 5.83 | 5.54 | 5.51 | 6.438 | 0.072 | −0.256 |
| 3-$ClC_6H_4$ | 5.64 | 5.45 | 5.39 | 6.121 | 0.072 | −0.256 |
| 4-$BrC_6H_4$ | 5.59 | 5.40 | 5.39 | 6.428 | 0.116 | −0.259 |
| 3,4-$Cl_2C_6H_3$ | 5.48 | 5.67 | 5.68 | 7.194 | 0.111 | −0.260 |
| 4-$ClC_6H_4$ | 5.16 | 5.24 | 5.22 | 6.109 | 0.133 | −0.260 |
| $C_6H_5$ | 4.83 | 5.05 | 4.97 | 5.041 | 0.074 | −0.256 |
| 4-$CH_3C_6H_4$ | 4.70 | 4.57 | 4.65 | 5.891 | 0.342 | −0.263 |
| 4-$FC_6H_4$ | 4.68 | 4.93 | 4.95 | 5.839 | 0.200 | −0.265 |
| 2-$ClC_6H_4$ | 4.56 | | | 6.114 | 0.133 | −0.260 |
| 3-$NO_2C_6H_4$ | 3.83 | 3.86 | 3.80 | 7.780 | 1.983 | +0.313[e] |
| 4-$NO_2C_6H_4$ | 3.70 | 3.67 | 3.66 | 7.999 | 2.065 | +0.306[e] |



| | | | | | | |
|---|---|---|---|---|---|---|
| 1-Naphthyl | 6.40 | 6.39 | 6.43 | 8.817 | 0.102 | −0.231 |
| 3-$ClC_6H_4$ | 5.80 | 5.69 | 5.40 | 6.139 | 0.066 | −0.258 |
| 3,4-$Cl_2C_6H_3$ | 5.77 | 5.86 | 5.69 | 7.212 | 0.107 | −0.262 |
| $C_6H_5$ | 5.30 | 5.34 | 4.98 | 5.059 | 0.067 | −0.258 |
| 2-$ClC_6H_4$ | 4.77 | | | 6.131 | 0.127 | −0.262 |
| 3-Indolyl | 4.41 | 4.40 | 5.02 | 8.909 | 0.594 | −0.312 |



| | | | | | | |
|---|---|---|---|---|---|---|
| $C_8H_5$ | 5.46 | 5.46 | 5.26 | 4.973 | 0.098 | −0.161 |
| 2-Thienyl | 3.60 | 3.60 | 3.10 | 7.620 | 1.323 | −0.247 |
| 3-Pyridyl | 3.22 | 3.22 | 4.20 | 4.641 | 0.502 | −0.161 |

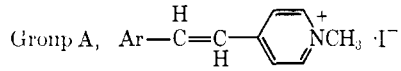
[a] Log of the reciprocal of the molar concentration which causes 50% *in vitro* inhibition of partially purified rat brain ChA.[1,2] [b] Sum of the calculated electrophilic superdelocalizabilities for all aryl atoms. [c] Sum of the calculated absolute $\pi$ charges for all aryl atoms. [d] Energy (in $\beta$ units)[4] of the lowest empty molecular orbital. [e] Other calculations performed on these molecules using adjusted HMO parameters ($h_n = h_0 = kN\text{-}O = kC\text{-}N = 1$)[12] gave more realistic $E_{LEMO}$ values of −0.259 and −0.148 for the 3-$NO_2$ and 4-$NO_2$ compounds, respectively. Use of these values in the correlations gave results which were slightly poorer, but equivalent, to the reported correlations.

Encouraging results were obtained by considering the HMO derived individual properties of each ring and the ethylene bridge. Correlations were attempted using such quantities as: $\Sigma S^E$, sum of electrophilic superdelocalizabilities; $\Sigma S^N$, sum of nucleophilic superdelocalizabilities; $\Sigma|Q|$, sum of absolute charges. $S^E$ can be related to $\pi$ donor ability and $S^N$ is related to $\pi$ acceptor ability (in both cases, the larger the value, the greater the donor or acceptor propensity). Absolute charge reflects polar or electrostatic properties of a region. The best results of these attempts are presented in Table II. It was possible to correlate the ChA inhibitory activity of the compounds in all three groups (separately) considering only the sums of the electrophilic superdelocalizabilities ($\Sigma S_{Ar}{}^E$) and absolute charges ($\Sigma|Q|_{Ar}$) of the *aryl portion* of the molecules (eq 1, 5, and 7).[7] The correlation was somewhat weaker when all compounds were considered (groups A, B and C) (eq 8). It has been suggested[2] that a 2-Cl substituent on the aryl portion of styrylpyridine-like compounds may detract from activity by decreasing coplanarity. Visual examination of the correlation data for groups A and B indicated that the 2-chloro derivative in each of these groups deviated most from the regression equation. The regressions for these groups were again performed excluding the 2-Cl derivatives (eq 2,6) and indeed the correlations improved. This technique also improved the correlation when all compounds were considered (eq 9), although it was still comparatively weak.

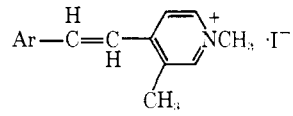
The correlation of variation in ChA inhibitory potency among styrylpyridine derivatives and analogs with $\Sigma S_{Ar}{}^E$ and $\Sigma|Q|_{Ar}$ suggests that two complementary

(7) Correlations could also be obtained by summing these quantities over the aryl and ethylenic atoms or over all atoms. However, the contribution of atoms other than the aryl group within each series was essentially constant. Thus, these correlations added unnecessary complexity and were neglected.

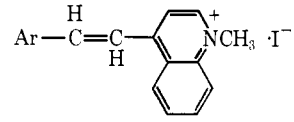
TABLE II
BEST HMO CORRELATION EQUATIONS

Group A, 

| | Equation | $r$[a] | $F$[b] | $n$[c] |
|---|---|---|---|---|
| (1) | $\log 1/I_{50} = 0.443\Sigma S_{Ar}^{E} - 1.170\Sigma\lvert Q\rvert_{Ar} + 2.610$ | 0.929 | 28.38 | 12 |
| | (all) $(4.15)_{c\ 99.5\%}$ $(7.53)_{99.99\%}$ | | (99.9%) | |
| (2) | $\log 1/I_{50} = 0.422\Sigma S_{Ar}^{E} - 1.190\Sigma\lvert Q\rvert_{Ar} + 2.816$ | 0.959 | 45.58 | 11 |
| | (−2Cl) $(4.92)_{99.8\%}$ $(9.55)_{99.99\%}$ | | (99.9%) | |
| (3) | $\log 1/I_{50} = 0.368\Sigma S_{Ar}^{E} - 2.713\Sigma\lvert Q\rvert_{Ar} + 5.405E_{LEMO} + 4.677$ | 0.944 | 21.85 | 12 |
| | (all) $(3.25)_{98\%}$ $(2.51)_{95\%}$ $(1.44)_{80\%}$ | | (99.9%) | |
| (4) | **$\log 1/I_{50} = 0.347\Sigma S_{Ar}^{E} - 2.732\Sigma\lvert Q\rvert_{Ar} + 5.402E_{LEMO} + 4.882$** | 0.974 | 42.88 | 11 |
| | (−2Cl) $(4.21)_{99.5\%}$ $(3.49)_{99\%}$ $(1.99)_{90\%}$ | | (99.9%) | |

Group B, 

| | | | | |
|---|---|---|---|---|
| (5) | $\log 1/I_{50} = 0.376\Sigma S_{Ar}^{E} - 4.215\Sigma\lvert Q\rvert_{Ar} + 3.506$ | 0.931 | 9.70 | 6 |
| | (all) $(2.98)_{90\%}$ $(4.39)_{95\%}$ | | (95%) | |
| (6) | $\log 1/I_{50} = 0.316\Sigma S_{Ar}^{E} - 4.091\Sigma\lvert Q\rvert_{Ar} + 4.016$ | 0.995 | 96.29 | 5 |
| | (−2Cl) $(7.87)_{98\%}$ $(13.85)_{99\%}$ | | (97.5%) | |

Group C, 

| | | | | |
|---|---|---|---|---|
| (7) | $\log 1/I_{50} = 1.350\Sigma S_{Ar}^{E} - 4.435\Sigma\lvert Q\rvert_{Ar} - 0.818$[d] | 1.000 | 114,250 | 3 |
| | (all) (367.5) ($\infty$) | | | |
| | All ChA Inhibitors | | | |
| (8) | $\log 1/I_{50} = 0.332\Sigma S_{Ar}^{E} - 1.298\Sigma\lvert Q\rvert_{Ar} + 3.317$ | 0.829 | 19.79 | 21 |
| | (all) $(3.37)_{99.5\%}$ $(6.21)_{99.999\%}$ | | (99.9%) | |
| (9) | $\log 1/I_{50} = 0.321\Sigma S_{Ar}^{E} - 1.330\Sigma\lvert Q\rvert_{Ar} + 3.465$ | 0.850 | 20.79 | 19 |
| | (−2Cl's) $(3.27)_{99.5\%}$ $(6.38)_{99.999\%}$ | | (99.9%) | |
| (10) | $\log 1/I_{50} = 0.389\Sigma S_{Ar}^{E} - 2.288\Sigma\lvert Q\rvert_{Ar} + 3.904E_{LEMO} + 4.098$ | 0.907 | 26.21 | 21 |
| | (all) $(4.98)_{99.9\%}$ $(7.15)_{99.999\%}$ $(3.59)_{99.5\%}$ | | (99.9%) | |
| (11) | $\log 1/I_{50} = 0.377\Sigma S_{Ar}^{E} - 2.309\Sigma\lvert Q\rvert_{Ar} + 3.864E_{LEMO} + 4.232$ | 0.925 | 29.85 | 19 |
| | (−2Cl's) $(5.08)_{99.9\%}$ $(7.61)_{99.999\%}$ $(3.75)_{99.8\%}$ | | (99.9%) | |

[a] $r$ = correlation coefficient; strength of correlation. [b] $F$ = significance of correlation coeff; ( ) = confidence level. [c] $t$ test; significance of individual term; % indicates confidence level. [d] Although the high $r$, $F$, and $t$ values suggest an excellent correlation here, the small sample size makes this impossible to verify. However, good correlation of $\log 1/I_{50}$ for this series with partition values calculated as in ref 8a suggests that the former correlation is valid. [e] $n$ = number of compounds.

and interrelated types of interactions may be occurring between the aryl moiety and the enzyme. It has been shown[8a,b] that the partition coefficient is a function of $\Sigma S^{E}$ and $\Sigma|Q|$ for a variety of aromatic systems. Thus, hydrophobic bonding between the aryl moiety and the enzyme appears important. Further, the relationship between $S^{E}$ and $\pi$ donor properties suggests that both charge transfer and hydrophobic interactions may be occurring between the aryl moiety and ChA.[9] These interpretations support and clarify the role of the aryl moiety envisioned in the working hypothesis.

In a search for better correlations applicable to all of the compounds and to examine the relevance of the $\pi$ acceptor postulate made earlier, additional terms were evaluated in the regression equations.[10] The only such term which improved the correlations in a statistically significant manner was $E_{LEMO}$ (eq 3,10). Again, deletion of the 2-Cl compound(s) improved the correlations (eq 4,11); the correlation for all styrylpyridine-like compounds (eq 11) improved greatly ($r$ = 0.925; $F$ = 96.29, 97.5% confidence level).

The significant contribution of $E_{LEMO}$ to ChA inhibitory activity implies that the $\pi$ acceptor properties of these compounds are also important to enzyme binding. This lends credence to the second part of the working hypothesis, although it cannot be categorically stated on this basis whether the whole molecule or only the pyridinium-like moiety is acting as the $\pi$ acceptor. Comparison of $\Sigma S^{N}$ values for the pyridinium-like moiety with $\Sigma S^{N}$ values for the aryl moiety does suggest that the pyridinium portion is at least more strongly involved.

At this point it became of interest to determine whether application of the Hansch technique would yield results in support of, or at variance with, the conclusions derived from the HMO treatment. Theoretical basis exists[8a,b; 11a,b] for the interrelation of such methods.

The Hansch method was applied to compounds of

(8) (a) K. S. Rogers and A. Cammarata, *J. Med. Chem.*, **12**, 692 (1969); (b) K. S. Rogers and A. Cammarata, *Biochem. Biophys. Acta*, **193**, 22 (1969).

(9) A visual examination of trends in the HMO data suggests that when Ar is Ph or substituted Ph, the $\pi$ donor component may predominate through positions *ortho* and *para* to the ethylenic moiety.

(10) Insufficient compound variations were available to permit treatment of groups B and C on an individual statistically significant basis.

(11) (a) A. Cammarata, *J. Med. Chem.*, **11**, 1111 (1968); (b) A. Cammarata, *ibid.*, **12**, 324 (1969).

TABLE III
BEST HANSCH CORRELATIONS
Group A (minus 2-Cl derivative)

| | Equation | $r^a$ | $F^b$ | $n^d$ |
|---|---|---|---|---|
| (12) | $\log 1/I_{50} = 1.245\sigma - 3.208\sigma^2 - 0.037\pi + 1.185\pi^2 + 4.668$ | 0.973 | 26.60 | 11 |
| | $(1.56)_{c\,80\%}$ $(2.86)_{95\%}$ $(0.05)_{<20\%}$ $(1.99)_{90\%}$ | | (99.9%) | |
| (13) | $\log 1/I_{50} = 1.725\pi - 0.807\sigma + 4.297$ | 0.905 | 18.02 | 11 |
| | $(5.90)_{99.9\%}$ $(1.94)_{90\%}$ | | (>99.5%) | |
| (14) | $\log 1/I_{50} = 1.216\pi - 1.657E_{LEMO} + 4.080$ | 0.948 | 35.43 | 11 |
| | $(4.94)_{99.8\%}$ $(3.61)_{99\%}$ | | (99.9%) | |

[a] $r$ = correlation coefficient; strength of correlation. [b] $F$ = significance of correlation coeff; ( ) = confidence level. [c] $t$ test; significance of individual term; % indicates confidence level. [d] $n$ = number of compounds.

group A (stilbazole derivatives). This group was selected as the aryl group of *all* of these compounds was a phenyl or substituted phenyl for which literature values[6] of $\pi$ were available; the 2-chloro derivative was not treated. The procedures and parameters utilized are discussed in the Experimental Section.

The Hansch analysis provided additional support for the conclusions derived from the HMO calculations (see Table III). The complete Hansch equation $(\sigma,\sigma^2,\pi,\pi^2)$(eq 12) afforded a good correlation of ChA inhibitory activity. Examination of the $t$ tests for individual terms in the equation, however, showed several of these to be relatively insignificant. The reason for this was clarified by the demonstration that linear relations exist between $\sigma$ and $\sigma^2$ ($r$ = 0.913) and between $\pi$ and $\pi^2$ ($r$ = 0.966); such relations develop when the $\sigma$ (or $\pi$) data set contains terms of predominantly one sign.[12] In such cases, the validity of correlations with $\sigma^2$ or $\pi^2$ is questionable at best.[12] An equation containing simply $\pi$ and $\sigma$ (eq 13) was thus generated and also provided a good correlation of the data. The contribution of $\pi$ suggests (as did the HMO results) that hydrophobic interactions are involved in binding of these compounds to ChA; the $\sigma$ term implies that some electronic factors are also involved.

In an effort to determine what electronic factors (represented by $\sigma$) were contributing to ChA binding, various HMO derived quantities were introduced in place of $\sigma$. Such procedures have met with success in correlating biological activities of other compounds.[13] The only such term that contributed in a statistically significant manner was $E_{LEMO}$. Introduction of this term with $\pi$ provided a good correlation of ChA inhibitory activity (eq 14) in agreement with the HMO results (eq 6,11). The facile replacement of $\sigma$ with $E_{LEMO}$ suggests that in the case of these compounds, the $\sigma$ term in eq 13 reflects *primarily* the electronic effects of substituents on $E_{LEMO}$. Notably, eq 4 and 14 represent the first demonstration *in a biological or biochemical system* of a relationship between $\pi$ and $\Sigma S^E$ and $\Sigma|Q|$.

In summary, the HMO and Hansch calculations performed on various styrylpyridine derivatives and analogs are consistant with the view that these compounds bind to ChA *via* hydrophobic and $\pi$ donor contributions of the aryl moiety and $\pi$ acceptor interactions, presumably by the pyridinium-like portion. No specific contribution (other than the previously suggested ones of facilitating coplanarity and conjugative transmission)[2] could be ascribed to the ethylenic linkage. Variation in activity among these compounds thus can be accounted for in quantitative terms using $\Sigma S_{Ar}{}^E$, $\Sigma|Q|_{Ar}$, and $E_{LEMO}$. For high potency a compound of this type in general should possess a high $\Sigma S_{Ar}{}^E$, a low $\Sigma|Q|_{Ar}$, and a small negative (or positive) $E_{LEMO}$.

## Experimental Section

The HMO calculations were performed on an IBM 360-75 computer through the University of North Carolina Computation Center, Chapel Hill, N. C. A program (70) supplied by the Indiana University Quantum Chemistry Program Exchange (Q.C.P.E.), modified by G. L. C. and R. C. A., was utilized. Parameters suggested by Streitweiser[14] were used; the ring carbons $\alpha$ to the quaternary N were assigned $h_e$ = 0.2.[15] Me groups attached to ring carbons were treated by the conjugated model. The Me on the quaternary N was neglected as it is a common denominator among these compounds. Further, it has been shown[1,2] that quaternary substituents in this series are nonspecific structural variables. The S of the thienyl derivative was treated as $h_s$ = 0, $k_{c-s}$ = 0.8.[4]

Hansch correlations and correlation of the HMO-derived values with activities were performed using a standard multiple regression program (UNCSTAT). Hansch $\pi$ values were those derived from the phenoxyacetic acids;[6] a value of 1.24 (for 2-naphthyl) was used as an approximation for the 1-naphthyl compound. A value of or 0.98 0.67 ($\pi_{3Cl} + \pi_{4Cl}$) was used as an approximation for $\pi_{3\text{-Cl},4\text{-Cl}}$. The $\sigma$ values used were taken from Hine;[16] the value used for the 2,3-$(CH)_4$ group (*e.g.*, the 1-naphthyl derivative) of 0.49 was calculated using the $pK_a$.[16,17]

**Acknowledgments.**—We are indebted to the University of North Carolina Computation Center for use of their facilities and to the University for financial support. The assistance of the Indiana University Quantum Chemistry Program Exchange in supplying the HMO program is acknowledged. We wish to thank Dr. Arthur Cammarata of Temple University for helpful comments and suggestions.

(12) A. Cammarata, personal communication.

(13) W. B. Neely, H. C. White, and A. Rudzik, *J. Pharm. Sci.*, **57**, 1176 (1968).

(14) Reference 4, p 135.

(15) R. D. Brown and R. D. Harcourt, *J. Chem. Soc.*, 3451 (1959).

(16) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1962, p 87.

(17) C. D. Hodgman, R. C. Weast, and S. M. Selby, Ed., "Handbook of Chemistry and Physics," 42nd ed, Chemical Rubber Co., Cleveland, Ohio, 1960–1961, p 1755.