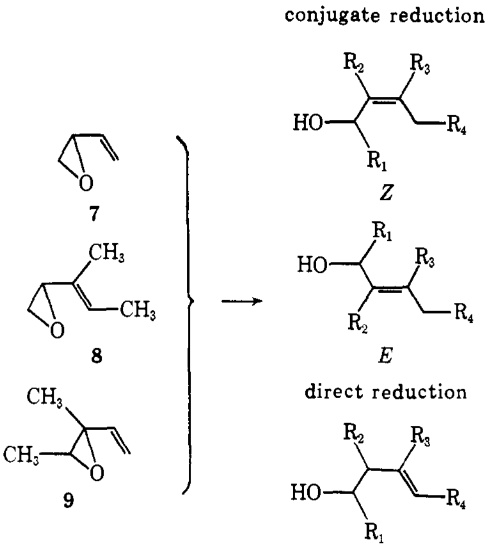
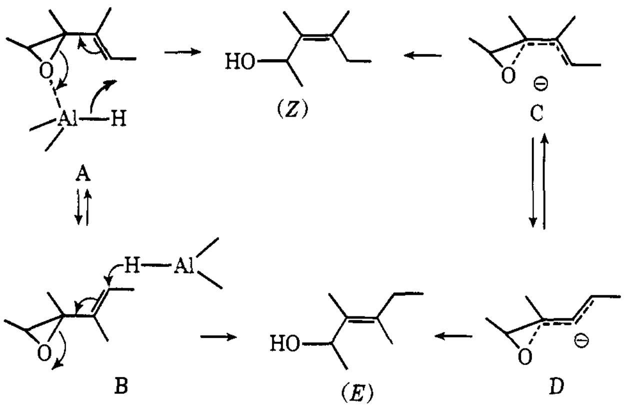
isomer **2** (Table I). Similarly, reduction of **1** with either lithium or sodium metal in liquid ammonia gave **3** in higher yield but with reduced stereoselectivity.[10]

With the results from the aforementioned system in mind, we have investigated several other $\alpha,\beta$-unsaturated epoxide systems to see if analogous reductions could be carried out when the substitution pattern on the unsaturated epoxide was varied. To this end, the reductions of 1,2-oxido-3-butene (**7**),[11] (*E*)-3-methyl-1,2-oxido-3-pentene (**8**),[12] and 3-methyl-2,3-oxido-4-pentene (**9**)[13] with DIBAH in refluxing hexane and with lithium and calcium metal dissolved in liquid ammonia were studied. The results are summarized in Table II, with data from system **1** included for comparison.



Comparison of the reactions of these four systems can best be made by considering first the extent to which reduction occurs in a conjugate, as opposed to a direct, fashion, and then the degree to which the conjugate reduction is stereoselective. Systems **1** and **9** have substituents that hinder the oxirane function, but none that hinder the double bond; reduction of both these systems with the three reagents studied proceeds cleanly in a conjugate fashion. System **7**, which is hindered at neither function, experiences predominantly conjugate reduction, but more so with the dissolving metals than with DIBAH. System **8**, with the double bond quite severely hindered but the oxirane accessible, undergoes about 50% direct attack on the epoxide function with all three reagents. Thus, the course of the reduction, conjugate *vs.* direct, seems in these systems to be simply a function of the relative degree to which the two functional groups are hindered, conjugate reduction being somewhat more favored with the dissolving metal reagents than with DIBAH.

The stereoselectivity of the conjugate reduction with the two dissolving metals favors the *E* isomer and is very high in all four systems, calcium generally being more selective than lithium. With DIBAH, the stereoselectivity favors the *Z* isomer but is much more variable; it is high in systems **1** and **7**, but low in **8** and **9**, and does not appear to depend upon either the overall hindrance of the system or the ease with which the system undergoes conjugate reduction.



It is tempting to speculate on the mechanistic factors that result in the opposing stereoselectivities of the DIBAH and dissolving metal reductions. With DIBAH in nonpolar medium, the aluminum may be internally solvated by the epoxide oxygen. Delivery of hydride within this complex could be accomplished only in that rotamer (A) having the vinyl group positioned syn to the hydride source, to give the *Z* isomer; the minor isomer could result from hydride delivery by uncomplexed reagent to the other rotamer (B). With the dissolving metal reductions, negative charge in the transition state is most likely distributed over the four atoms; the more extended conformer D, which minimizes charge repulsion, would be stabler, so that the *E* isomer would predominate.

Work is currently in progress on the extension of this reaction to other systems and application to the synthesis of certain natural products.

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[10] These dissolving metal reductions are quite similar to a recently described reaction of an unsaturated cyclic oxide: D. Joulain and F. Rouessac, *J. Chem. Soc. D*, 314 (1972).

[11] Commercially obtained material was used (Columbia Chemical Co.).

[12] Prepared from the corresponding bromohydrin synthesized according to the method of D. R. Dalton and R. M. Davis, *Tetrahedron Lett.*, 1057 (1972).

[13] Prepared from *trans*-3-methyl-1,3-pentadiene and *m*-chloroperbenzoic acid in methylene chloride.

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## Unusual Polarographic Behavior of Arylaziridinium Salts[1]

*Sir:*

Previous polarographic studies of aziridinium ions (generated *in situ*) emphasized either quantitative analysis[2a,b] or the nature of the reduction process.[2c–f]

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[1] Presented in part at the 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 28, 1972, Abstracts of Papers, ORGN-17.

[2] (a) N. G. Lordi and J. E. Christian, *J. Amer. Pharm. Ass.*, **45**, 530 (1956); (b) R. Mantsavinos and J. E. Christian, *Anal. Chem.*, 30, 1071 (1958); (c) A. Anhalt and H. Berg, *J. Electroanal. Chem.*, **4**, 218 (1962), and references therein; (d) E. Bauer and H. Berg, *Chem. Zvesti*, **18**, 454 (1964); (e) B. Jambor, I. P. Horvath, and L. Institoris, *Magy. Kem. Foly.*, **73**, 332 (1967); *Chem. Abstr.*, 67, 102840 (1967); (f) D. A. Tyssee and M. M. Baizer, *J. Electrochem. Soc.*, **118**, 1420 (1971).

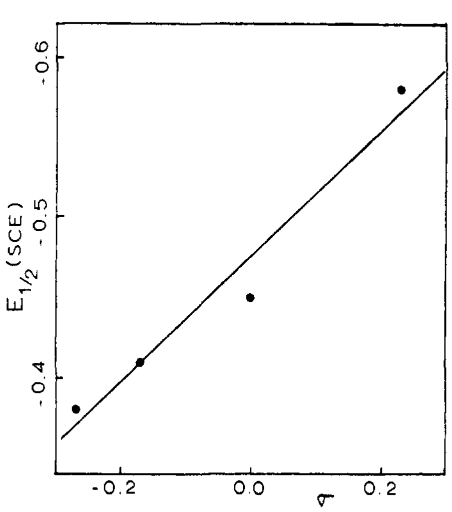
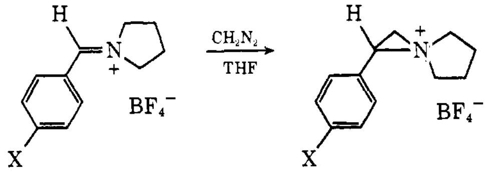


Figure 1. Modified Hammett plot of half-wave potentials of arylaziridinium salts **2a–d**.

Recently techniques have been developed for isolation of aziridinium salts, and this has led to a better understanding of the reactivity of this class of compounds.[3] We now report our results on the first systematic study relating aziridinium salt structure to electrochemical reduction, and in particular the unusual substituent effect on half-wave potentials ($E_{1/2}$).

Arylaziridinium salts **2a–d** were prepared in the



**1a**, X = Cl
**b**, X = H
**c**, X = $CH_3$
**d**, X = $OCH_3$

**2**

manner reported by Keenan and Leonard.[4] Structures were assigned on the basis of elemental analyses, absence of ir absorptions corresponding to iminium or N–H bonds, and pmr spectra which showed the expected upfield shifts (relative to the iminium salts) of protons on the three- and five-membered rings. Alternative piperazinium salt structures could be ruled out on the basis of pmr data (insufficient absorption in the $\delta$ 4–6 region[5]), solubility and melting point correlations,[5] and reactivity toward solvolysis and reduction.

All aziridinium salts gave well-defined polarographic waves at $5 \times 10^{-4}$ *M* in water with a Borax buffer (pH 6.8)[6] as the supporting electrolyte. A nonlinear plot of log $(i/i_l - i)$ *vs.* $-E$ for **2b** showed that the electrode process was not reversible, with a $E_{1/4} - E_{3/4}$ value of 0.175 V. A linear correlation of $i_l$ with $h^{1/2}$ ($r$ = 0.999) indicated a diffusion-controlled process[7] corresponding to a two-electron wave.[8] Double layer capacitance, measured on an AC polarograph using *iR* compensation and phase selective detection,[9] was lower than that for the supporting electrolyte alone, suggesting possible adsorption of aziridinium salts.[10] Solvolysis did not interfere with the determination of the $E_{1/2}$ values given in Table I, although for **2d** a low initial current indicated that considerable solvolysis had occurred before the polarogram could be recorded. For reference, $E_{1/2}$ values of the iminium salts are also listed in Table I, but these undoubtedly represent the corresponding benzaldehydes formed on hydrolysis.

**Table I.** Structural Effects on Half-Wave Potentials of Iminium and Aziridinium Salts[a]

| | $E_{1/2}$, V (sce) | | |
|---|---|---|---|
| Substituent | Iminium salts **1** | ArCHO[b] | Aziridinium salts **2** |
| $p$-$OCH_3$ | −1.39 | −1.38 | −0.38 |
| $p$-$CH_3$ | −1.36 | −1.35 | −0.41 |
| H | −1.30 | −1.28 | −0.45 |
| $p$-Cl | −1.28 | −1.26 | −0.58 |

[a] All solutions $5 \times 10^{-4}$ *M* in pH 6.8 Borax buffer. [b] Authentic commercial samples.

For iminium salts (or benzaldehydes) electron attracting groups facilitate reduction, as reflected in more positive $E_{1/2}$ values and in agreement with polarographic data for benzylidine iminium salts in nonaqueous solvents.[11] This trend is entirely expected in view of nearly all of the reported reductions of benzene derivatives[12] in which the electron, as a nucleophilic species,[13] reacts more readily at more electrophilic sites.

In marked contrast to this result, electron attracting groups *hinder* reduction of arylaziridinium ions as seen by more negative $E_{1/2}$ values and the modified Hammett plot (Figure 1), which gives a negative reaction constant of −0.39 V ($r$ = 0.979). Reduction of aziridinium salts is thus one of only a few well-substantiated examples of benzene derivatives which exhibit negative reaction constants.[13b,14] These cases, which include reduction of nitrobenzenes in sulfuric acid[14,15] and, for electron releasing groups, reduction of benzyl bromides[16] and benzene diazonium ions,[17] appear to involve formation of a net positive charge in proceeding from the ground state to the electron transfer step.

The abnormal substituent effect for aziridinium salts can be interpreted in a similar way as formation of a partial positive charge at the benzylic carbon before

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(3) (a) N. J. Leonard, *Rec. Chem. Progr.*, **26**, 211 (1966); (b) D. R. Crist and N. J. Leonard, *Angew. Chem.*, **81**, 953 (1969); (c) D. R. Crist and N. J. Leonard, *Angew. Chem., Int. Ed. Engl.*, **8**, 962 (1969).

(4) T. R. Keenan and N. J. Leonard, *J. Amer. Chem. Soc.*, **93**, 6567 (1971).

(5) N. J. Leonard and J. A. Klainer, *J. Heterocycl. Chem.*, **8**, 215 (1971).

(6) H. T. S. Britton, "Hydrogen Ions," Vol. 3, 2nd ed, Van Nostrand, New York, N. Y., 1931, p 222.

(7) L. Meites, "Polarographic Techniques," 2nd ed, Interscience, New York, N. Y., 1965, pp 132, 218.

(8) Calculated using a value of $6 \times 10^{-6}$ cm$^2$/sec for $D$ in the Ilkovic equation, ref 7, p 117.

(9) R. de Levie and J. C. Kreuser, *J. Electroanal. Chem.*, **21**, 221 (1969).

(10) (a) R. de Levie and J. C. Kreuser, *ibid.*, **38**, 239 (1972); (b) B. Breyer, *Progr. Polarogr.*, **2**, 492 (1962).

(11) C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.*, **26**, 223 (1970).

(12) P. Zuman, "Substituent Effects in Organic Polarography," Plenum Press, New York, N. Y., 1967, pp 58–67.

(13) (a) C. L. Perrin, *Progr. Phys. Org. Chem.*, **3**, 292 (1965); (b) ref 12, pp 97–99.

(14) C. L. Perrin, *Progr. Phys. Org. Chem.*, **3**, 293 (1965).

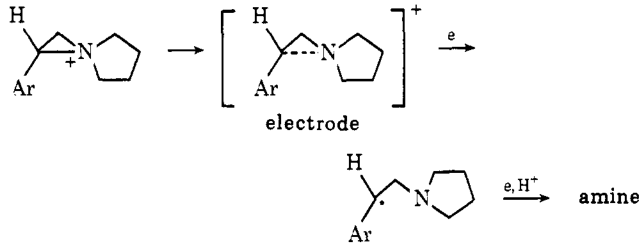
(15) (a) J. C. James, *Trans. Faraday Soc.*, **47**, 1240 (1951); (b) P. Zuman, *Advan. Polarogr.*, **3**, 812 (1960).

(16) G. Klopman, *Helv. Chim. Acta*, **44**, 1908 (1961).

(17) J. K. Kochi, *J. Amer. Chem. Soc.*, **77**, 3208 (1955).

electron transfer, as represented in Scheme I. On the

**Scheme I**



basis of reported work on the $N,N$-dialkylaziridinium salts[2f] and benzyl bromides[16] it seems reasonable to assume two one-electron transfers.[18] This mechanism is consistent with the observed two-electron wave, since in water the radicals produced are more easily reduced than the reactant.

An alternate process involving complete formation of an aminocarbonium ion in a prior equilibrium step is considered less likely at the present time, since $E_{1/2}$ values correlate better with $\sigma$ values than with $\sigma^+$. However, this point is being investigated by measuring $E_{1/2}$ values in aprotic solvents in which the combined wave may be separable.

The present electrochemical results can be compared to other ring opening reactions of aziridinium ions. It was recently reported that 1-ethyl-1-azoniabicyclo-[3.1.0]hexane perchlorate (which cannot form tertiary or benzylic carbonium ions) reacts with nucleophiles exclusively by an S$_N$2 mechanism.[19] However, 2-aryl systems react with water[20] or benzaldehyde[4] *via* an aminocarbonium ion, the same type of species that is reduced by nucleophilic electrons at an electrode surface.

**Acknowledgment.** The authors express their appreciation to Dr. V. Horak for most useful discussions and to Dr. R. de Levie who helped us perform AC measurements on his instrument. The generous support by the Research Corporation is gratefully acknowledged.

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(18) We thank one of the referees for suggesting this process.

(19) C. F. Hammer, S. R. Heller, and J. H. Craig, *Tetrahedron*, **28**, 239 (1972).

(20) N. B. Chapman and D. J. Triggle, *J. Chem. Soc.*, 1385 (1963).

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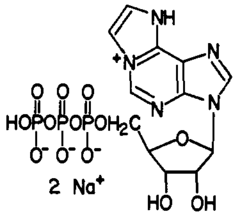
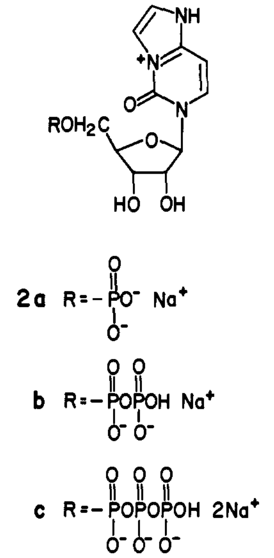
## Enzymatic Activity and $\gamma$-$^{32}$P Labeling of Fluorescent Derivatives of Cytidine Triphosphate and Adenosine Triphosphate

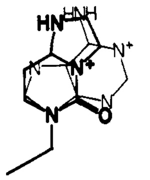
*Sir:*

We wish to report a fluorescent derivative of CTP that replaces ATP in certain enzymatic reactions. Chloroacetaldehyde reacts with cytidine to produce a 3,$N^4$-etheno-bridged derivative.[1] Similarly, we have prepared the corresponding derivatives of the cytidine ribonucleotides, namely, 3,$N^4$-ethenocytidine 5′-phosphate, or 5′-εCMP (**2a**, shown as the sodium salt), 3,$N^4$-ethenocytidine 5′-diphosphate (εCDP, **2b**), and 3,$N^4$-ethenocytidine 5′-triphosphate (εCTP, **2c**).[2] The 1,$N^6$-etheno derivatives of adenine nucleotides, *e.g.*, εATP (**1**, shown as the disodium salt), are known to replace the corresponding unmodified nucleotides in a number of enzymatic reactions.[3]



**1**





**3**

In the enzymatic phosphorylation of 3-phosphoglyceric acid, εCTP[2] is 1.4 × 10$^3$ times as active as CTP, is essentially equivalent to ATP, and is a significantly better coenzyme than εATP (**1**). This ability to replace ATP permits the enzymatic synthesis of [$\gamma$-$^{32}$P]εCTP and [$\gamma$-$^{32}$P]εATP by phosphate exchange.

Approximately 1.0–1.6 *M* aqueous chloroacetaldehyde[3d] was used in about 20-fold excess to convert the cytidine nucleotides to the derivatives with a 3,$N^4$-etheno bridge, by stirring at pH 3.5 at 22–37°, until no starting material was detected on cellulose thin layer chromatograms (Eastman Chromagram cellulose sheets using isobutyric acid–NH$_4$OH–H$_2$O, 75:1:24, v/v).[4] Decolorization with charcoal, evaporation to dryness followed by precipitation from aqueous solution with ethanol, and washing the precipitate with ethanol gave pure products.[5] The ε-cytidine nucleotides have fluorescent properties similar to those reported for the corresponding nucleoside[1b] and are indistinguishable in this respect. All show a fluorescence emission maximum at 347 nm under acidic conditions upon excitation at 280 nm. The ability of εATP and εCTP to phosphorylate 3-phosphoglyceric acid catalyzed by yeast 3-phosphoglycerate kinase was compared to that of ATP. The phosphorylation was assayed according to

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(1) (a) N. K. Kochetkov, V. N. Shibaev, and A. A. Kost, *Tetrahedron Lett.*, 1993 (1971); (b) J. R. Barrio, J. A. Secrist III, and N. J. Leonard, *Biochem. Biophys. Res. Commun.*, **46**, 597 (1972); (c) N. K. Kochetkov, V. N. Shibaev, and A. A. Kost, *Dokl. Akad. Nauk SSSR*, **205**, 100 (1972).

(2) In the shortened forms of the names, the abbreviation "ε" now generally in use stands for the etheno bridge and is also suggestive of the molar absorbance term and fluorescence emission.

(3) (a) J. A. Secrist III, J. R. Barrio, and N. J. Leonard, *Science*, **175**, 646 (1972); (b) J. A. Secrist III, J. R. Barrio, N. J. Leonard, C. Villar-Palasi, and A. G. Gilman, *Science*, **176**, 279 (1972); (c) J. R. Barrio, J. A. Secrist III, and N. J. Leonard, *Proc. Nat. Acad. Sci. U. S.* **69**, 2039 (1972); (d) J. A. Secrist III, J. R. Barrio, N. J. Leonard, and G. Weber, *Biochemistry*, **11**, 3499 (1972); (e) R. F. Steiner, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **23**, 139 (1972).

(4) $R_f$ values are: εCMP, 0.54; εCDP, 0.37; εCTP, 0.24 *vs.* CMP, 0.39; CDP, 0.18; CTP, 0.09.

(5) Satisfactory elemental analyses were obtained for all the new compounds mentioned (2a–c). If needed, chromatography on DEAE Sephadex using a gradient of ammonium formate (0.5–1.2 *M*) (pH ~3.7) produced excellent purification of the chloroacetaldehyde-modified cytosine derivatives.