Polarographic Study of New Antibacterial Isoxazolium Salts: 2,3-Dialkyl-5-arylisoxazolium Perchlorates

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Abstract □ The polarographic reduction of new antibacterial 2,3-dialkyl-5-arylisoxazolium salts is described. The electrode process involves an irreversible two-electron transfer and is pH independent within the 6.0–10.0 range. In more acidic media, hydrogen waves are observed; at higher pH values, a chemical degradation of the isoxazolium salt proceeds. Values of the half-wave potential of the salts are dependent on the polar contribution of the 5-aryl substituents in terms of the Hammett equation. The *in vitro* minimum inhibitory concentrations of these compounds are best correlated with Hansch π-values.

Keyphrases □ Isoxazolium salts, substituted—polarographic reduction, effect of pH, polar contribution of 5-aryl substituents correlated with antibacterial activity □ Polarography—reduction, substituted isoxazolium salts, effect of pH, polar contribution of 5-aryl substituents correlated with antibacterial activity □ Antibacterial activity—substituted isoxazolium salts, correlated with polar contribution of 5-aryl substituents, polarographic reduction □ Structure–activity relationships—substituted isoxazolium salts, polar contribution of 5-aryl substituents correlated with antibacterial activity, polarographic reduction

In a search for new antimicrobial cationic agents, the synthesis and antibacterial evaluation of a series of 2,3-dialkyl-5-arylisoxazolium perchlorates (I–XIII) were undertaken (1). Since differences in the antibacterial effectiveness of these salts were roughly assigned to substituent effects, it was of interest to evaluate such effects by tracing the possible electronic contributions of the isoxazolium 2- and 5-substituents and to correlate the corresponding microbiological data with these effects.

This report describes the polarographic reduction of I–XIII and its suitability as a measure of the polar substituent effect. Correlation of the antibacterial effectiveness to the polar substituent effect and the substituent solubility effect also was attempted.

EXPERIMENTAL

Materials—Analytically pure 2,3-dimethyl- and 2-ethyl-3-methyl-5-arylisoxazolium perchlorates (I–XIII) were prepared by quaternizing the appropriate isoxazoles as reported previously (2). Double-distilled water was used for all solutions.

Reagents—A Britton–Robinson phosphate–acetate–borate buffer was used. Adjustment to the required pH value was effected by addition of 0.2 *N* carbonate-free sodium hydroxide solution (3). Potassium chloride and tetraethylammonium perchlorate were utilized as the supporting electrolytes in 0.1–1.0 *M* solutions. Octoxynol[1] (0.1% v/v) and freshly prepared gelatin (0.2% w/v) solutions were the maximum suppressors. For deoxygenation, oxygen-free nitrogen was prepared by passing commercial nitrogen through 5% (w/v) aqueous vanadous sulfate solution (4).

Instrumentation—Current–voltage curves were recorded on a suitable polarograph[2] using 25-ml H-type polarographic cells, a dropping mercury electrode, and a saturated calomel electrode. Temperature control was achieved with an ultrathermostat[3], switched at 25° (±0.1).

Capillary characteristics were: drop time, 3.0 sec; and rate of mercury flow, 3.5 mg/sec, measured at a 30.0-cm mercury column height without applied potential. Recording conditions were: full scale deflection, 0.1 μamp; applied potential, 0.0–2.0 at a rate of 200 mv/min; and shunt, $\frac{1}{200}$. A pH meter[4] fitted with a sealed calomel electrode and a shielded glass electrode was used. For the spectrophotometric monitoring, a manual spectrophotometer[5] and 1-cm thick quartz cells were employed.

Procedure—The corresponding weight of the isoxazolium salt was dissolved in an appropriate volume of 0.5 *M* potassium chloride solution (or in a selected buffer solution), 0.5–1.0 ml of the maximum suppressor solution was added, and the solution was diluted with the supporting electrolyte solution to a final concentration of 5×10^{-4} *M* depolarizer/liter. Electrolysis was conducted at 25° after deoxygenation with pure nitrogen for 10 min.

RESULTS AND DISCUSSION

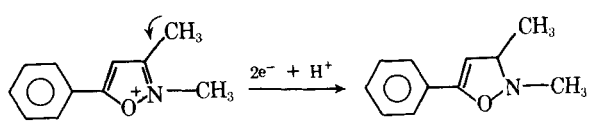
A literature review revealed that data pertaining to the polarographic reduction of the isoxazolium cation are not available, although the electroactivity of other 6π-isoelectronic analogs has been established (5–7). When tested for such activity in 0.5 *M* potassium chloride solution (pH 6.60–6.75), 2,3-dimethyl-5-phenylisoxazolium perchlorate (I) afforded a single well-defined cathodic wave with a half-wave value of −1.05 v (Fig. 1). This value was not significantly affected by variations in the ionic strength of the potassium chloride solution (0.10–2.0 *M*).

The use of 0.1 *M* aqueous tetraethylammonium perchlorate solution as the supporting electrolyte brought about a negative shift in the wave ($E_{1/2}$ = −1.12 v), reflecting the relatively high electrophilicity of the isoxazolium cation relative to its isosteric pyrazolium analog. The $E_{1/2}$ of 1,2,3-trimethyl-5-phenylpyrazolium perchlorate is −1.69 v (5).

As revealed from the dependence of the limiting current values of I, both on concentration (1×10^{-3}–5×10^{-4} *M*) and on the square root of the mercury column height (20–45 cm), its reduction wave is diffusion controlled. Comparison of the wave height and slope with relevant data of the similarly investigated pyrazolium analog gives the electron transfer of I a value of $n \sim 2$ (5). Theoretical logarithmic analysis of the observed wave gave rise to a straight line; however, the slope corresponded to a nonintegral value for n ($\alpha = 0.134$, $\alpha n \neq 0.059$), thus suggesting the irreversibility of the electrode reaction (8). Based on reported electrophilic interactions of the isoxazolium cation (9), the polarographic reduction of I might be similarly expected to afford the corresponding isoxazoline (Scheme I).

With the Britton–Robinson buffer as the supporting electrolyte, no apparent change in the wave characteristics of I could be traced within pH 6.0–10.0. At lower pH values (2.0–5.5), gradual reduction of the hydrogen ion seriously precluded the observation of the isoxazolium wave, while an increase of alkalinity (10.5–11.9) brought about a remarkable fall in the wave height. The $E_{1/2}$ value was little affected by such pH variations. At pH values higher than 11.9, a continous prominent drop of the limiting current was observed.

Estimations of the actual concentration of I at such pH values by UV spectrophotometric[6] and polarographic monitoring were similar. This finding denotes a considerable instability of the isoxazolium cation at elevated pH values, probably because of a base-induced cleavage of the cation (10). Since the antibacterial effectiveness of most cationic agents



Scheme I

[1] Triton-X 100.
[2] LP-60 A, Prague, Czechoslovakia.
[3] T-606 MTA, Budapest, Hungary.
[4] *Radelkis OP-401/2, Budapest, Hungary.*
[5] Spektromom-203, Budapest, Hungary.
[6] The 282-nm absorption maximum of I was not affected by variations in the buffer pH.

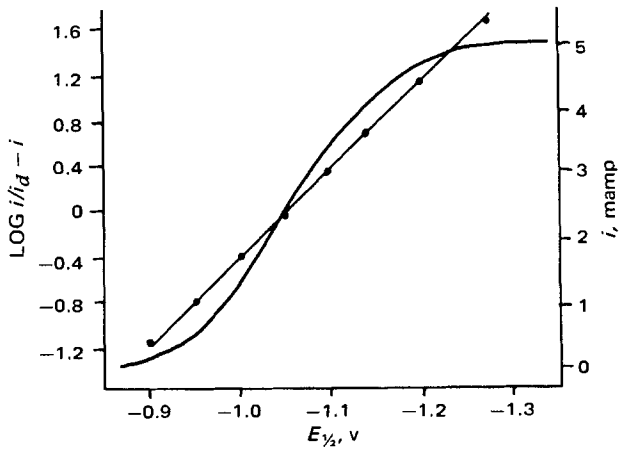


Figure 1—*Wave equation of I, 5 × 10⁻⁴ M in 0.5 M potassium chloride solution.*

is enhanced with increased pH values, further antimicrobial investigations of this series of isoxazolium salts will be carried out at pH values up to 10.0.

Polarographic reduction of II–XIII was carried out under the same conditions (Table I). Except for the two nitro derivatives, VII and XIII, comparison of the wave characteristics of the investigated salts (in 0.5 *M* potassium chloride) indicated that their electrode processes were identical to those of I. Compounds VII and XIII were reduced with the production of two waves; the first ($E_{1/2} \sim -0.68$ v) corresponded, however, to a four-electron transfer for the reduction of the nitro group (5). The second wave ($E_{1/2} \sim -1.28$ v) involved a two-electron reduction of the nitro-reduced isoxazolium cation.

The polar contribution of the 5-aryl substituents of I–XIII can be determined with the modified Hammett equation (11):

$$\Delta E_{1/2} = E_{1/2}^{x} - E_{1/2}^{0} = \sigma\rho \qquad \text{(Eq. 1)}$$

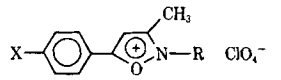
where $E_{1/2}^{x}$ and $E_{1/2}^{0}$ are the polarographic half-wave potentials of the benzene-substituted and unsubstituted derivatives of a particular series, respectively; ρ represents the reaction constant; and σ is the Hammett substituent constant. For I–VI and VIII–XII, correlation of their $E_{1/2}$ values to the corresponding 5-aryl σ-constants was fairly significant (r = 0.90 and 0.96, ρ = 0.26 and 0.28, and s = ±68 and ±46 mv for the *N*-methyl and *N*-ethyl sets, respectively).

The weak sensitivity of the polarographic reaction center of the investigated salts to the polar substituent effect was readily reflected by the relatively low values of the electrode reaction constant. Since such values are usually encountered when substituents are far away from reaction centers (12), this result would favor the representation of the isoxazolium 3-carbon for the polarographic reaction center (Scheme I).

The fact that substitution of the *N*-methyl radical for an *N*-ethyl analog did not affect the electrophilic tendency of the carrier isoxazolium cation (Table I) is explained by the electronic leveling effect of polar contributions of the *N*-methyl and *N*-ethyl moieties; σ-constants are −0.15 and −0.17, respectively (13). In contradiction to the reported polarographic data for related nitrophenyl heterocycles (5, 6), the $E_{1/2}$ values of the nitrophenylisoxazolium salts were not linearly correlated to their NHOH-substituent constant, probably due to a difference between their electrode reaction and other 5-arylisoxazolium derivatives.

Moreover, no correlations could be set up between the $E_{1/2}$ values of the investigated salts and their corresponding minimum inhibitory concentration (MIC) values. Added to the fact that VIII–XIII are more microbiologically effective than their *N*-methyl congeners (I–VII), these data suggest a weak operation of the polar contributions of both of the 2- and 5-isoxazolium substituents. In fact, the correlation of logarithmic MIC values of I–VII to the corresponding Hansch partition values of the

Table I—Antibacterial Activity and Substituent Constants of 2,3-Dialkyl-5-arylisoxazolium Perchlorates



Compound	R	X	$E_{1/2}$, v	σX^- Constant[a]	πX^- Value[a]	MIC, μg/ml[b]
I	CH_3	H	−1.050	0.00	0.00	800
II	CH_3	CH_3	−1.070	−0.17	+0.56	300
III	CH_3	OCH_3	−1.085	−0.27	−0.02	400
IV	CH_3	OC_2H_5	−1.075	−0.24	+0.38	300
V	CH_3	Cl	−0.960	+0.23	+0.71	200
VI	CH_3	Br	−0.940	+0.27	+0.86	150
VII	CH_3	NO_2	−1.275[c]	+0.78	−0.28	1000
VIII	C_2H_5	H	−1.045	0.00	0.00	500
IX	C_2H_5	CH_3	−1.050	−0.17	+0.56	200
X	C_2H_5	OCH_3	−1.075	−0.27	−0.02	250
XI	C_2H_5	OC_2H_5	−1.070	−0.24	+0.38	120
XII	C_2H_5	Br	−0.960	+0.27	+0.86	100
XIII	C_2H_5	NO_2	−1.280[c]	+0.78	−0.28	600

[a] From Ref. 13. [b] Minimum inhibitory concentration against *Staphylococcus aureus*. [c] Another wave existed at $E_{1/2}$ = −0.675 v.

5-phenyl substituents (π-values, Table I) was fairly significant (r = 0.97 and r^2 = 0.94 for n = 7). Such data also favor interpretation of the increased effectiveness of the *N*-ethylisoxazolium congeners in terms of their enhanced lipophilicity, π-CH_3 and C_2H_5 = +0.56 and +1.02, respectively (13).

REFERENCES

(1) N. M. Omar and A. A. El-Kader, *Can. J. Pharm. Sci.*, **8**, 128 (1973).

(2) C. Munsante, *Gazz. Chim. Ital.*, **72**, 448, 537 (1942).

(3) U. U. Lyrie, "Handbook of Analytical Chemistry," Khimia, Moscow, Russia, 1967, p. 230.

(4) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).

(5) N. M. Omar and N. A. El-Rabbat, *J. Pharm. Sci.*, **63**, 937 (1974).

(6) Z. N. Timoofeeva, N. M. Omar, L. S. Tikhonova, and A. V. Eltsov, *Zh. Obshch. Khim.*, **40**, 2072 (1970).

(7) Z. N. Timoofeeva and A. V. Eltsov, *ibid.*, **37**, 2599 (1967).

(8) I. K. Kolthoff and J. J. Lingane, "Polarography," vol. I, Interscience, New York, N.Y., 1965, p. 120.

(9) A. Ikou, H. Kaoru, and K. Heido, *Tetrahedron Lett.*, **55**, 4875 (1969).

(10) R. B. Woodward and R. Olofson, *J. Am. Chem. Soc.*, **83**, 1007 (1961).

(11) P. Zuman, *Collect. Czech. Chem. Commun.*, **19**, 599 (1954).

(12) U. A. Jdanov and V. I. Minkin, "Correlation Analysis in Organic Chemistry," Khimia, Moscow, Russia, 1968, p. 134.

(13) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

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