Polarographic Study of New Antimicrobial Pyrazolium Salts I: 1,2,5-Trimethyl-3-arylpyrazolium Perchlorates

NABIL M. OMAR[x] and NAWAL A. EL-RABBAT

Abstract □ The polarographic reduction of a new series of antimicrobial pyrazolium salts is described. Effects of the 3-aryl substituents on the $E_{1/2}$ values of the corresponding salts are well correlated with Hammett σ constants. The electrode reaction is shown to be mediated by a two-electron transfer and is pH independent. The irreversible electron transfer seems to be the rate-limiting factor of the electrode process. The *in vitro* minimum inhibitory concentrations of the title compounds against *Staphylococcus aureus* do not give a linear correlation with the corresponding $E_{1/2}$ values.

Keyphrases □ 1,2,5-Trimethyl-3-arylpyrazolium perchlorates—polarographic reduction, $E_{1/2}$ values correlated with antimicrobial minimum inhibitory concentrations □ Pyrazolium salts, antimicrobial—polarographic reduction of 1,2,5-trimethyl-3-arylpyrazolium perchlorates, structure-activity correlations □ Antimicrobial agents, new pyrazolium salts—polarographic study □ Polarography—reduction of new antimicrobial pyrazolium salts, effects of 3-aryl substituents on $E_{1/2}$ values

Many pyrazole derivatives have been reported to act as potent antimicrobial agents. Among these, the 1,2,5-trimethyl-3-arylpyrazolium salts (I) are characterized by marked antifungal effects (1, 2). The order of antibacterial activity of these compounds is appreciably influenced by the nature of the 3-aryl substituent. The pyrazolium ion was recently reported to undergo electrochemical reduction at the dropping mercury electrode (3); therefore, it was of interest to evaluate possible polar effects of both 3- and 5-substituents on the polarographic behavior of the pyrazolium ring and to correlate such effects with the *in vitro* antibacterial activity of these compounds.

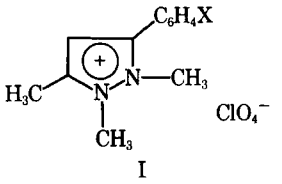
EXPERIMENTAL

1,2,5-Trimethyl-3-arylpyrazolium Perchlorates (I–VIII)—The salts were prepared according to reported methods (1) and were evaluated by UV spectrophotometry for purity.

Polarographic Reduction of Compounds I–VIII—The corresponding weight of the pyrazolium salt was dissolved in 50 ml double-distilled water containing 1.15 g polarographically pure tetraethylammonium perchlorate and 1.25 ml 0.2 % freshly prepared gelatin solution so as to produce a final concentration of 5×10^{-4} *M* depolarizer/liter. The polarograms were recorded on a polarograph[1], using an H-type cell, dropping mercury electrode, and saturated calomel electrode, after deoxygenation with oxygen-free nitrogen for 20 min at 25 ± 0.5°.

Capillary Characteristics—The drop time (*t*) was 3 sec, and the mass of the drop (*m*) was 3.5 mg/sec, measured at 30 cm height (*h*) and without applied voltage. The mercury used was of a polarographic double-distilled grade.

[1] LP-60.



I

Recording Conditions—The full-scale deflection (fsd) was 0.1 μamp, the applied voltage (v) was 0–2 in 200 mv/min, and the sensitivity shunt (S) was 1/200 fsd.

RESULTS AND DISCUSSION

The electrochemical reduction of the investigated pyrazolium salts proceeded successfully only when a supporting electrolyte of a highly negative reduction potential, like tetraethylammonium perchlorate, was used. Trials to adopt supporting electrolytes such as potassium chloride, Britton–Robinson buffer, or acetate buffer solutions resulted in the production of hydrogen waves at potentials more positive than that required for the reduction of the investigated salt. In 0.1 *M* aqueous tetraethylammonium perchlorate solution (pH 6.2–6.4), 1,2,5-trimethyl-3-phenylpyrazolium perchlorate readily affords a single cathodic wave with $E_{1/2}$ of −1.69 v (Fig. 1). As revealed from the dependence of the limiting current value, both on the concentration of the depolarizer and on the mercury column height (*h*), this wave is purely diffusion controlled. Theoretical logarithmic analysis of the wave (log $i/i_d - i$ *versus* *E*, Fig. 1) gives rise to a straight line, the slope of which corresponds, however, to a nonintegral value for *n* (α = 0.077, $\alpha n \neq 0.059$), thus denoting the irreversibility of the electrode reaction.

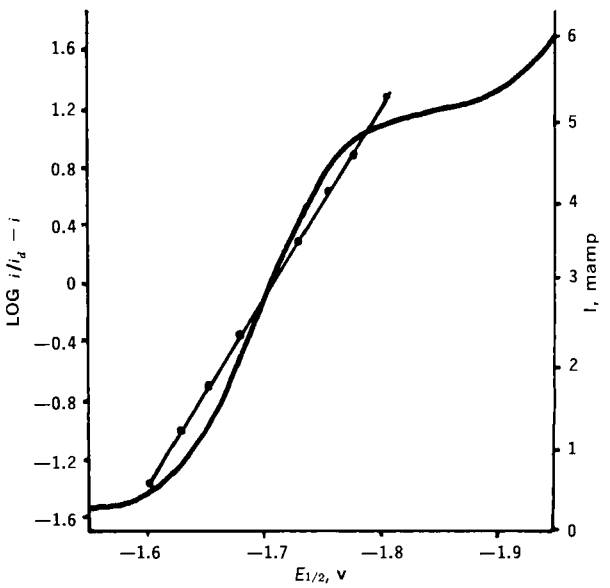


Figure 1—*Wave equation of 1,2,5-trimethyl-3-phenylpyrazolium perchlorate, 5×10^{-4} M in 0.1 M tetraethylammonium perchlorate.*

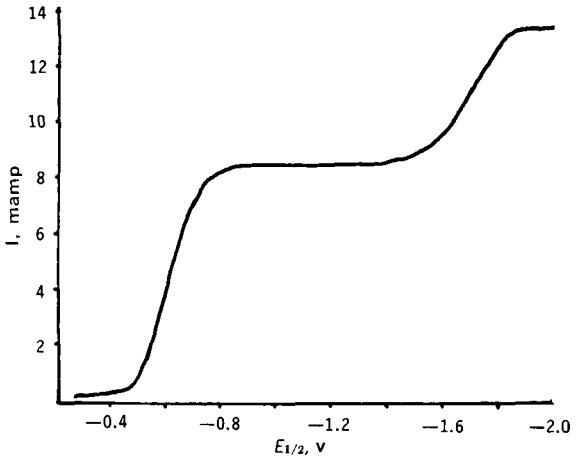


Figure 2—*Polarographic reduction of 5 × 10⁻⁴ M 1,2,5-trimethyl-3-(m-nitrophenyl)pyrazolium perchlorate in 0.1 M aqueous tetraethylammonium perchlorate.*

The number of the electrons involved in the reduction of Compound I, $n \cong 2$, is determined from the application of the Ilkovič equation (4). Essential for this determination is the diffusion coefficient of the pyrazolium ion (D_{0+}: 1.05×10^{-5} cm²/sec), computed from the limiting equivalent conductance (λ^0) of Compound I determined at 25°.

Controlled macroelectrolysis as well as chemical reduction of the 5-demethylated analogs of Compounds I–VIII afforded the corresponding 3-pyrazolines (3, 5). Accordingly, the two-electron polarographic reduction of I in protic media can be represented as shown in Scheme I.

Further study of the effect of the pH variation of the aqueous solutions of I on its polarographic behavior revealed that both the limiting current and the half-wave potential are only slightly, if at all, affected by the change of pH within a range of 6.2–13.0 (resulting from the controlled addition of sodium hydroxide). At pH values <6.2, a considerable decrease of the hydrogen overpotential is observed. Hence, in the polarographic reduction of I, protonation of the pyrazolium ion cannot be considered as the potential-determining stage.

The polarographic reduction of II–VIII is accomplished under the same exact conditions mentioned for I. The corresponding $E_{1/2}$ values (*versus* the saturated calomel electrode) are listed in Table I.

As revealed from a comparison of the wave heights of II–VIII, the electrode processes of these salts (except VIII) involve a two-electron transfer typical of that already cited. Moreover, the polarogram of VIII contains two reduction waves with $E_{1/2}$ values of −0.6 and −1.71, respectively (Fig. 2).

The peculiar polarographic behavior of VIII can be attributed to the presence of the polarographically active nitro group substituted in the 3-phenyl ring. Analysis of the corresponding heights of these two waves shows that the two-electron cathodic wave of the pyrazolium ion is preceded by a four-electron reduction of the

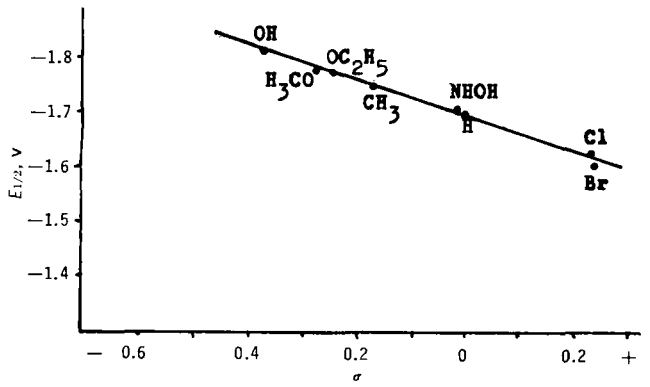


Figure 3—*Application of the Hammett equation to the half-wave potentials of I–VIII.*

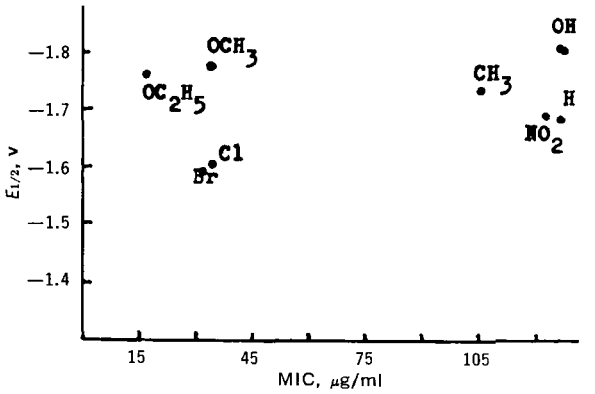


Figure 4—*Application of the Hammett equation to the MIC's of I–VIII against* S. aureus *in micrograms per milliliter.*

m-nitro group into the hydroxylamine derivative. This behavior is frequently reported for many aromatic compounds, where the nitro groups are acting principally *via* a −mesomeric effect (6–8).

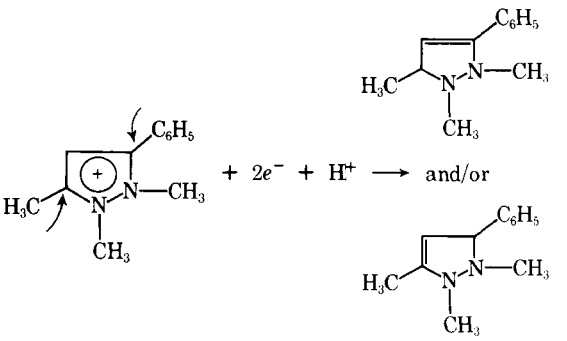
The quantitative determination of the polar effect of the different phenyl substituents of I–VIII, as well as the assignment of their electrode mechanisms, can be followed through the application of the principle of linear free energy, initially represented by the Hammett equation and recently extended to organic polarography in the form of:

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

in which $E_{1/2}$ and $E_{1/2}{}^0$ are the half-wave potentials of the substituted and unsubstituted derivatives of a particular series, respectively; σ is the Hammett substitution constant; and ρ is the reaction constant (9). For the investigated pyrazolium salts, the plot of the corresponding $E_{1/2}$ values *versus* their 3-phenyl substitution constants affords a linear relationship (Fig. 3).

Statistical analysis of this linearity using the least-squares method gave a significant correlation with the following characteristics: $r = 0.99$, $\rho = 0.33$ *V*, and $s = 0.005$. The fact that values for σ, and not those for σ^+, are successfully adopted in this correlation clearly demonstrates the localization of a relatively small positive charge over the reaction center of the heterocycle. This can be accepted as a direct result of the influence of the 5-methyl group, acting by both + inductive and + mesomeric effects on the adjacent pyrazolium C-5, which can be considered as the reaction center. Moreover, the nucleophilic nature of the electrode reaction is distinctly revealed from the positive value of ρ. Thus, the rate of electron transfer is considered the potential-determining step in the reduction of the investigated ions, the electron affinity of which (reflected upon the $E_{1/2}$ values) is markedly promoted by the presence of electron-attracting substituents (10). Consequently, removal of the 5-methyl group out of the structure of I–VIII facilitated the electrochemical reduction of the corresponding demethylated pyrazolium salts (3).

Since use of the Hammett equation has also been extended to structure–activity data of some biologically active organic compounds (11), it was of interest to correlate the *in vitro* minimum inhibitory concentrations (MIC) of the studied pyrazolium salts against *Staphylococcus aureus* (Smith), determined in Ref. 1,



Scheme I

Table I—Half-Wave Potentials of 1,2,5-Trimethyl-3-arylpyrazolium Perchlorates (II–VIII)

	Compound						
	II	III	IV	V	VI	VII	VIII
X	p-CH_3	p-OCH_3	p-OC_2H_5	p-OH	p-Cl	p-Br	m-NO_2
$E_{1/2}$	−1.75	−1.77	−1.77	−1.82	−1.63	−1.60	−0.6, −1.71

with the corresponding structural parameters. As indicated from the scattered results relating the MIC of the pyrazolium salt to the $E_{1/2}$ value (Fig. 4), the microbiological data of these compounds do not correspond with their polarographic behavior. This can be probably attributed to the existence of biological pathways other than those concerned with the reduction potential of the investigated compounds. In fact, the application of the Hammett equation in such biological fields is still limited to a small group of organic compounds.

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Comparison among Four Vehicles and Four Routes for Administering Δ^9-Tetrahydrocannabinol

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Abstract □ The effect of (−)-Δ^9-*trans*-tetrahydrocannabinol (I) on latency to hindlimb tonic extension produced by maximal electroconvulsive shock was studied in mice following injection by four routes of administration: oral, subcutaneous, intraperitoneal, and intravenous. For each route, four different vehicles were used: bovine serum albumin, polysorbate 80, polyvinylpyrrolidone, and propylene glycol. The anticonvulsant effect of I was strongest in the propylene glycol vehicle with subcutaneous and intraperitoneal routes and equal in the propylene glycol and polyvinylpyrrolidone vehicles with the oral route. With these three routes, bovine serum albumin and saline appeared to be inadequate vehicles for studying the anticonvulsant activity of I. When the intravenous route was used, anticonvulsant activity of I was found with all four vehicles, with propylene glycol and polyvinylpyrrolidone allowing the greatest effect of I; however, the propylene glycol vehicle itself also showed anticonvulsant activity. A further experiment showed that the duration of action of I following oral administration was substantially longer in the propylene glycol than in the polyvinylpyrrolidone vehicle.

Keyphrases □ Tetrahydrocannabinol—effect on hindlimb tonic extension, four vehicles and four routes of administration compared, mice □ Marijuana—comparison of four vehicles and four routes of administration, hindlimb tonic extension, mice □ Vehicles—effect on tetrahydrocannabinol anticonvulsant activity, comparison of four vehicles and four routes of administration, hindlimb tonic extension, mice

Recently, a report (1) from this laboratory described a study with mice which compared four vehicles for intraperitoneal administration of (−)-Δ^9-*trans*-tetrahydrocannabinol[1] (I), the apparent major psychoactive constituent of marijuana. The bioassay for an effect of I was latency to hindlimb tonic extension in mice following a maximal electroconvulsive shock. The most suitable vehicle for injection of I was found to be 10% propylene glycol–1% polysorbate 80[2]–0.9% saline. Other vehicles compared were 3% polyvinylpyrrolidone (2), 5% bovine serum albumin (3), and 1% polysorbate 80 (4), all in 0.9% sa-

[1] An alternative name for the same compound is Δ_1-tetrahydrocannabinol, using the pyran instead of the formal numbering system.

[2] Tween 80.