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# One-Electron Redox Reactions of Pyrazolin-5-ones

## A Pulse Radiolysis Study of Antipyrine and Analogues

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SUMMARY

One-electron oxidation of several derivatives of pyrazolin-5-one, including the drug antipyrine, were studied by pulse radiolysis of aqueous solutions. All the compounds were found to be oxidized by $Br_2^-$ rapidly ($k \sim 3 \times 10^8$–$2 \times 10^9$ $M^{-1}$ $s^{-1}$) but considerably more slowly by weaker oxidants, such as peroxyl radicals. From redox equilibria using *p*-methoxyphenol and *N,N,N′,N′*-tetramethyl-p-phenylenediamine as reference compounds, the one-electron oxidation potentials of the methyl-substituted 2-pyrazolin-5-ones were found to be in the range of 0.32–0.39 V *versus* normal hydrogen electrode. The relevance of these findings to the properties of the drug nafazatrom is discussed. Antipyrine was found to have a much higher oxidation potential, estimated as 1.2–1.5 V, which is rationalized on the basis of the phenyl substitution and lack of resonance stabilization of the radical cation.

### INTRODUCTION

Antipyrine, an anti-inflammatory agent, and nafazatrom, an antithrombotic and antimetastatic agent, are both derivatives of pyrazolin-5-one, and their biological activity may result from the reactivity of the pyrazolin-5-one moiety. In the case of nafazatrom (3-methyl-[2-(2-naphthyloxy)ethyl]-2-pyrazolin-5-one) it has been hypothesized that its activity is due to its reactivity with oxidizing radicals (1), a reactivity which has been confirmed in recent kinetic experiments (2). The reactivity of nafazatrom was postulated to be associated with the pyrazolin-5-one moiety. In the consideration of further development of pyrazolin-5-one-based drugs, it is important not only to obtain information on the reactivity of these compounds with oxidizing radicals but also to understand the effect of alkyl substitution on kinetics parameters. For this purpose we have carried out a pulse radiolysis study of several methyl-substituted pyrazolin-5-ones and their derivatives and have found a considerable effect of substitution in the 3- or 4-position. We have found also that the redox behavior of antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) is considerably different than that of the other derivatives studied.

### MATERIALS AND METHODS

Pyrazolin-5-one derivatives were obtained from Aldrich.[2] Nafazatrom was a generous gift from Dr. L. J. Marnett of Wayne State University. Except for 4-methyl-2-pyrazolin-5-one, which was purified by recrystallization from water, all other derivatives were used without further purification. p$K_a$ values for pyrazolin-5-ones were determined spectrophotometrically and are listed in Table 1 together with the structural formulas of the derivatives. Other chemicals were of the highest purity available and were used without further purification. *p*-Methoxyphenol and promethazine were obtained from Sigma, and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride was from Fisher. Water was purified by a Millipore Milli-Q system, and solutions were freshly prepared before each experiment. The pH was adjusted where necessary using sodium hydroxide or maintained using phosphate and borate buffers.

The pulse radiolysis experiments were conducted on the Febetron 705 pulse radiolysis set-up, described elsewhere (3). Doses/pulse were in the range of 2–50 gray, as determined by thiocyanate dosimetry.

### RESULTS AND DISCUSSION

One-electron oxidation of pyrazolin-5-ones, PyrH, can be achieved, as shown for nafazatrom (2), by the reaction with $Br_2^-$ radicals. Pulse radiolysis experiments were carried out first to determine the rate constants for the oxidation of the various derivatives. Solutions containing 0.1 M $Br^-$ and various pyrazolin-5-one concentrations ($0$–$5 \times 10^{-4}$ M) at different pH values were $N_2O$ saturated and then irradiated with electron pulses. Under these conditions $Br_2^-$ radicals are generated (4) in less than 0.1 μs. Kinetic spectrophotometric measurements showed the immediate formation of $Br_2^-$ after the pulse ($\lambda_{max}$ = 360 nm), followed by the decay of this radical on the millisecond time scale. The reactivity of the PyrH derivatives with $Br_2^-$

$$Br_2^- + PyrH \rightarrow \cdot Pyr + H^+ + 2Br^- \qquad (1)$$

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[2] The mention of commerical products does not imply recommendation or endorsement by the National Bureau of Standards nor does it imply that the products identified are necessarily the best available for the purpose.

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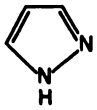
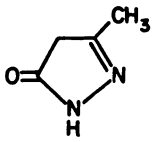
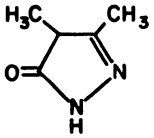
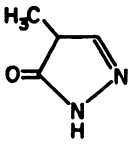
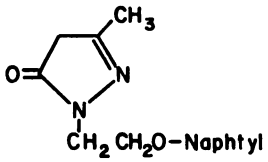
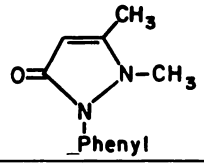
TABLE 1

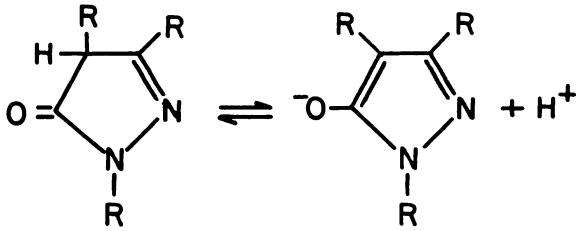
*Reactivities of oxidizing radicals with pyrazolin-5-ones*

| Derivative | | $pK_a$ | Oxidizing radical | pH | $k$[a] |
|---|---|---|---|---|---|
| | | | | | $M^{-1}\,s^{-1}$ |
| Pyrazole |  | 11.5[b] | $Br_2^-$ | 8.8 | $<10^6$ |
| 3-Methyl-2-pyrazolin-5-one |  | 8.9 | $Br_2^-$ | 8.8 | $7.0 \times 10^8$ |
| 3,4-Dimethyl-2-pyrazolin-5-one |  | 8.9 | $Br_2^-$ | 6.6 | $5.6 \times 10^8$ |
| | | | | 8.8 | $9.2 \times 10^8$ |
| | | | | 12.4 | $13.0 \times 10^8$ |
| | | | $CCl_3O_2^-$ | 7.0 | $4.3 \times 10^7$ |
| 4-Methyl-2-pyrazolin-5-one |  | 8.9 | $Br_2^-$ | 8.8 | $7.9 \times 10^8$ |
| Nafazatrom |  | 9.2[c] | $Br_2^-$ | 9.2 | $1.0 \times 10^{9c}$ |
| Antipyrine[b] |  | | $Br_2^-$ | 7–12 | $3.3 \times 10^8$ |

[a] Estimated to be accurate to ±10%.
[b] From Ref. 11.
[c] From Ref. 2.

was monitored at 360 nm. From the effect of pyrazolin-5-one concentration on the rate of decay of $Br_2^-$, the second order rate constants for Reaction 1 were determined and are summarized in Table 1.

The reaction rate constants for $Br_2^-$ with pyrazolin-5-ones were found to be pH dependent (Fig. 1) due to the ionization of PyrH



(2)

The $pK_a$ values for the derivatives are shown in Table 1. The reactivities of 3-methyl, 4-methyl, and 3,4-dimethyl-2-pyrazolin-5-one are very similar. On the other hand, antipyrine is oxidized somewhat less rapidly, probably due to electron withdrawing by the phenyl substituent. This substitution also prevents deprotonation, and thus the reactivity of antipyrine remains low even at higher pH. Pyrazole itself could not be oxidized by $Br_2^-$ since it is a fully aromatic molecule, unlike pyrazolin-5-ones whose ring is partially saturated

We also attempted to oxidize dimethylpyrazolin-5-one with the more weakly oxidizing peroxyl radical $CCl_3O_2^-$ produced in air-saturated $CCl_4$ solutions (5). The rate constant was found to be more than an order of magnitude lower than that for $Br_2^-$. However, since the lifetime of peroxyl radicals in general is longer than that of $Br_2^-$, the present result indicates that 2-pyrazolin-5-ones would have a high probability of scavenging peroxyl radicals in biological systems.

The optical absorption spectra of the radicals produced by oxidation of pyrazolin-5-ones with $Br_2^-$ were similar. These radicals have absorption maxima around 320–330 nm (Fig. 2) similar to the spectrum of the nafazatrom radical (2). This supports the previous premise that oxidation of nafazatrom occurs on the pyrazolin-5-one moiety. Antipyrine oxidation leads to a radical absorbing also at 330 nm, indicating oxidation of the pyrazolin-5-one ring.

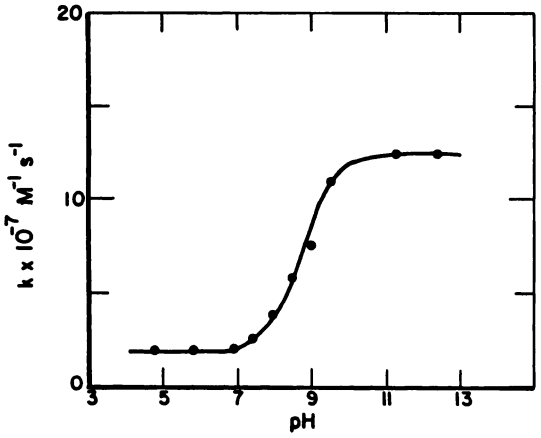



FIG. 1. *pH dependence of the rate constant for the $Br_2^-$ radical reaction with 4-methyl-pyrazolin-5-one*

Full line is drawn through the experimental points assuming the inflection point at pH 8.7.

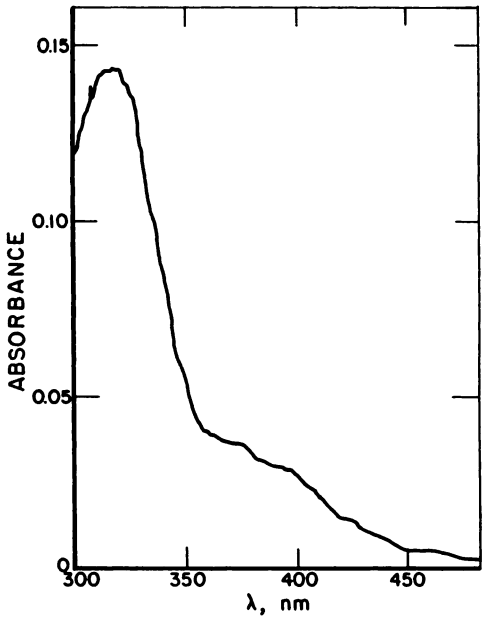



FIG. 2. *Absorption spectrum of the radicals produced by oxidation of 4-methyl-pyrazolin-5-one*

5 mM 4-methyl-pyrazolin-5-one, 0.1 M KBr, 1 atm $N_2O$, dose/pulse = 50 gray, 20°.

The results discussed thus far point to the similarity between various pyrazolin-5-one derivatives in their rate constant with $Br_2^-$ and site of oxidation. In the biological systems, however, where the oxidizing radicals may be of different varieties and more weakly oxidizing, the efficiency of a compound in scavenging some or all of these radicals will be greatly determined by its redox potential. We have attempted, therefore, to measure one-electron oxidation potentials of the various pyrazolin-5-ones. For this purpose we chose *p*-methoxyphenol and TMPD[3] as redox reference compounds whose potentials are known (6). These compounds, like pyrazolin-5-ones, are rapidly oxidized by $Br_2^-$ to an extent which is determined by their reactivity and concentration relative to those of the pyrazolin-5-one with which they compete. After this initial rapid oxidation, a slower process is observed which involves electron transfer leading to equilibrium, *e.g.*,

$$p\text{-}CH_3OC_6H_4O^{\cdot} + Pyr^- \rightleftharpoons p\text{-}CH_3OC_6H_4O^- + Pyr^{\cdot} \quad (3)$$

This equilibrium was found to be achieved before any substantial decay of the radicals. Therefore, an equilibrium constant can be determined, as shown before (6) from the concentrations (absorbances) or from the kinetics, which is then used to calculate the redox potential. The results are summarized in Table 2.

It is seen from Table 2 that 4-methyl- and 3,4-dimethyl-pyrazolin-5-one are oxidized by *p*-methoxyphenoxyl radical with $k_f = 5 \times 10^7$ and $1 \times 10^8$ $M^{-1}$ $s^{-1}$, respectively, $k_r$ being in both cases about 20 times slower. From these kinetics, and from the absorbances, the redox potentials for these two pyrazolin-5-ones were found to be very similar, 0.32 and 0.33 V *versus* normal hydrogen electrode. The 3-methyl derivative, on the other hand, was not found to be oxidized by *p*-methoxyphenoxyl with a measurable rate constant, and neither was the back reaction observable. Therefore, its redox potential is probably very close to that of *p*-methoxyphenol (0.40 V). Experiments with TMPD as a reference exhibited the expected reactions and allowed the determination of a redox potential of 0.39 V for 3-methyl-2-pyrazolin-5-one, indeed very similar to that of *p*-methoxyphenol.

This pronounced difference between the redox poten-

TABLE 2

*Redox properties of pyrazolin-5-ones*

$p\text{-}CH_3OC_6H_4O^{\cdot} + Pyr^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} p\text{-}CH_3OC_6H_4O^- + Pyr^{\cdot}$

| | $k_f$[a] | $k_r$[a] | $K_{kin}$[b] | $K_{abs}$[c] | $E^1_{12.2}$[d] |
|---|---|---|---|---|---|
| | | | | | V |
| 4-Methyl-2-pyrazolin-5-one | $5.2 \times 10^7$ | $2.5 \times 10^6$ | 20 | 11 | 0.32 |
| 3,4-Dimethyl-2-pyrazolin-5-one | $9.6 \times 10^7$ | $5.4 \times 10^6$ | 18 | 12 | 0.33 |
| 3-Methyl-2-pyrazolin-5-one | $<10^5$ | $<10^5$ | | | [e] |
| Nafazatrom | $<5 \times 10^5$ | | | | [e] |
| Antipyrine | Slow[f] | $2.7 \times 10^9$ | $>10^3$ | | >0.6 |
| $Pyr^{\cdot} + TMPD \rightleftharpoons Pyr^- + TMPD^+$ | | | | | |
| 3-Methyl-2-pyrazolin-5-one | $2.4 \times 10^6$ | $2.8 \times 10^4$ | 84 | 150 | 0.39 |

[a] Rate constants in units $M^{-1}$ $s^{-1}$, measured at pH 12.2, accurate to ±10% for the reactions proceeding in the favorable direction and ± 20% for others.

[b] Equilibrium constant determined from the kinetics, accurate to ± 20%.

[c] Equilibrium constant determined from the absorbances at equilibrium.

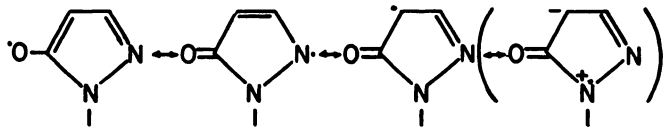
[d] Calculated from $\Delta E = 0.059 \log K$ using the average of both $K$ values and $E^1_{12.2}$ for *p*-methoxyphenol (0.40 V), or for TMPD (0.27 V) *versus* normal hydrogen electrode, estimated to be accurate to ±0.01 V.

[e] See "Results and Discussion."

[f] The reverse reaction proceeds to completion.

[3] The abbreviation used is: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

tials of the 4-methyl and 3,4-dimethyl derivatives on the one hand and that of the 3-methyl-2-pyrazolin-5-one on the other hand can be rationalized by the difference in the effects of the methyl substitution in the 3- and 4-positions. Based on the ESR spectrum of the nafazatrom radical it was concluded that the spin density was highest on O, N2, and C4 (2).



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Therefore, methyl substitution on C4 will result in hyperconjugation with the delocalized electron on the radical and thus lower the redox potential, while methyl substitution on C3 will have only a minimal effect due to the low spin density on C3.

Based on this interpretation, nafazatrom should have a redox potential similar to that of 3-methyl-pyrazolin-5-one, *i.e.*, ~0.39 V. Indeed, we have found that this drug is not readily oxidized by the *p*-methoxyphenoxyl radical. Methyl substitution on C4 of nafazatrom will enhance its oxidizability and may thus enhance its biological activity, to the extent that the latter is dependent on the redox potential.

Antipyrine radical cation was found to oxidize *p*-methoxyphenol rapidly and quantitatively, indicating that its redox potential is >0.6 V (Table 2). Therefore, we attempted to measure its potential by using other reference systems. We found that the antipyrine radical oxidizes promethazine ($E^1 = 0.86$ V (7, 8)) rapidly ($k = 1.6 \times 10^9$ $M^{-1}$ $s^{-1}$) and quantitatively at pH 7, *i.e.*, its redox potential is >1 V. Furthermore, we examined the possible oxidation of antipyrine by $(SCN)_2^-$, which is a weaker oxidant than $Br_2^-$ (1.25 V (9) *versus* 1.69 V (10)) and found no observable reaction. We conclude, therefore, that the one-electron oxidation potential of antipyrine must be in the range of 1.2–1.5 V, *i.e.*, almost 1 V higher than those of the other pyrazolin-5-ones studied. This large difference may be partially ascribed to electron withdrawing by the phenyl group. A more important factor, however, is likely to be the lack of deprotonation of this compound or its radical. Oxidation by $Br_2^-$ was found to be somewhat slow and must form the following cation radical.

$$Br_2^- + \text{Pyr-}\phi \rightarrow 2\ Br^- + \text{Pyr-}\phi^+ \qquad (4)$$

The inability of the cation radical to deprotonate, as it does in the other derivatives, probably prevents its further stabilization through resonance structures I–III and thus increases the redox potential, *i.e.*, reduces its antioxidant efficiency.

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