Kinetic Spectrophotometric Study of Effect of Triazolopyrazines on *p*-Benzoquinone Complexes

G. H. MOXON and M. A. SLIFKIN ˣ

Abstract □ The effect of adding triazolopyrazines to a *p*-benzoquinone–proline system and a *p*-benzoquinone–histamine system was studied by observing the kinetics of the spectral changes and analyzing the results using digital simulation methods. Below 35°, the major effect was that the triazolopyrazine competed with the electron donors for the acceptors to form complexes. Above 35°, there was predominantly a chemical interaction between the triazolopyrazine and the acceptors. A separate experiment showed that the triazolopyrazine interacted with *p*-benzoquinone to form a complex initially but that a chemical interaction took place later.

Keyphrases □ *p*-Benzoquinone complexes—with proline and histamine, effect of various triazolopyrazines, kinetic spectrophotometric study □ Triazolopyrazines, substituted—effect on complexes of *p*-benzoquinone with proline and histamine, kinetic spectrophotometric study □ Complexes—*p*-benzoquinone with proline and histamine, effect of various triazolopyrazines, kinetic spectrophotometric study □ Spectrophotometry—kinetic study, effect of triazolopyrazines on complexes of *p*-benzoquinone with proline and histamine □ Structure–activity relationships—various triazolopyrazines, effect on complexes of *p*-benzoquinone with proline and histamine

The spectral properties of some nitrogen heterocyclic drugs, some triazolopyrazines, and a triazolopyrimidine (1) have been studied, as has the effect of these drugs on the potentiation of the reversal of metachromasia of a toluidine blue–heparin complex in the presence of amino acids (2). It was suggested that the triazolopyrazines might function as electron donors, competing with both the dye and amino acids for acceptor sites on the dye. The electron-donating properties of some of these drugs have now been studied by observing the interaction directly with the electron acceptor *p*-benzoquinone and by observing the effect of the drugs on systems of *p*-benzoquinone with the electron acceptors histamine and proline.

Both systems were studied extensively by the method of time-dependent spectrophotometry under various temperature, solvent, and pH conditions. The curves were analyzed using digital simulation methods (3, 4). Furthermore, since some of these heterocyclic drugs have antiasthmatic activity, their interaction with a histamine system might have medicinal interest. The alteration of a reaction rate by the complexing of one reactant in a two-molecule system with an added third compound has been studied (5, 6) and is a very useful technique where the interaction between the added third compound and either interactant is weak or is not shown clearly by the techniques in use.

EXPERIMENTAL

Histamine, *p*-benzoquinone, and proline were obtained and purified as described previously (3, 4). 3-Amino-6-methyl-8-propyl-*s*-triazolo[4,3-*a*]pyrazine (I), 3-acetamido-5-methyl-8-*n*-propyl-*s*-triazolo[4,3-*a*]pyrazine (II), 3-acetamido-6-methyl-8-*n*-propyl-*s*-triazolo[4,3-*a*]pyrazine (III), and 2-amino-5-*n*-propyl-*s*-triazolo[2,3-*c*]-pyrimidine (IV) were used as supplied[1]. (Their structures are shown in Fig. 1 of Ref. 1.) The experimental methods and techniques were described previously (3, 4).

[1] Courtesy of ICI Pharmaceutical Division.

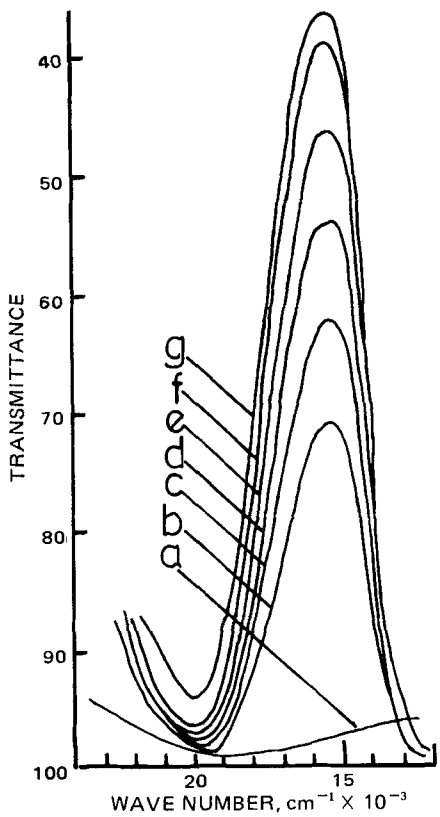


Figure 1—*Development of peak at 15,200 cm^{-1} (658 nm) attributed to the I (2 × 10^{-2} M)-p-benzoquinone (2 × 10^{-4} M) complex at 25° in pH 7 phosphate buffer. Readings were at: (a) zero time, (b) 6 days, (c) 7 days, (d) 8 days, (e) 9 days, (f) 10 days, and (g) 15 days.*

RESULTS AND DISCUSSION

Interaction of I with p-Benzoquinone—The effect of I with *p*-benzoquinone in phosphate buffer was studied first. Figure 1 shows the spectrum obtained in a mixed solution of the drug and quinone in pH 7 phosphate buffer. The effect of drug concentration on the development of the absorption spectrum is illustrated in Fig. 2. The shape of the curve is indicative of the formation of more than one species (7) and indicates that the interaction is of the form:

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_3} C$$

The observed peak at 15,200 cm^{-1} (658 nm) corresponds not to the immediate product of the interaction but to some subsequent reaction product (*C*), as evidenced by the initial induction period and the shape of its growth curve.

Figure 3 shows the effect of temperature on the absorption band at 15,200 cm^{-1} (658 nm). Increasing the temperature from 25 to 55° resulted in a marginal increase in the intensity which was not reversible. This behavior indicates a chemical interaction and not a complexing mechanism. The initial reaction between the drug and the quinone, which was not observable spectroscopically, probably is the formation of a reversible complex, since many organic amines and amides are known to form complexes with quinones and then chemical products (3, 8).

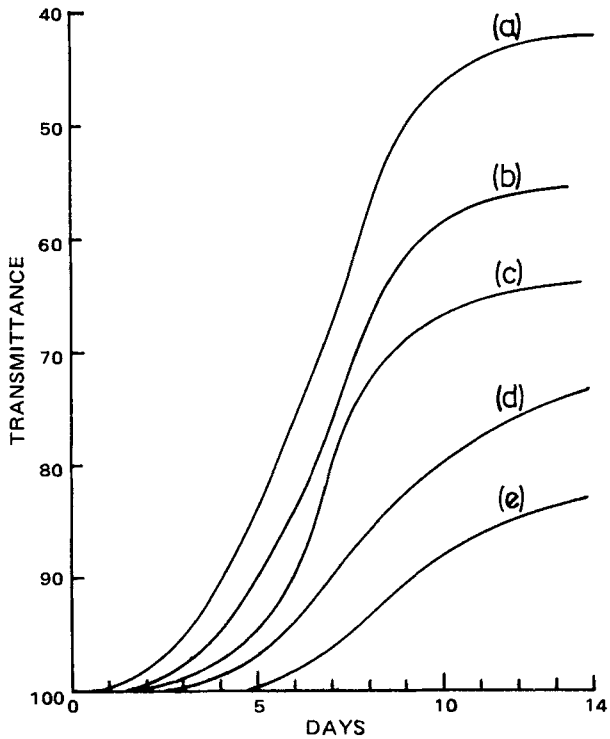


Figure 2—*Effect of I concentration on the development of the 15,200-cm^{-1} (658-nm) peak of the* p*-benzoquinone–I complex at 25° in pH 7 phosphate buffer. Key: a, 2 × 10^{-2} M; b, 10^{-2} M; c, 7 × 10^{-3} M; d, 4 × 10^{-3} M; and e, 10^{-3} M.*

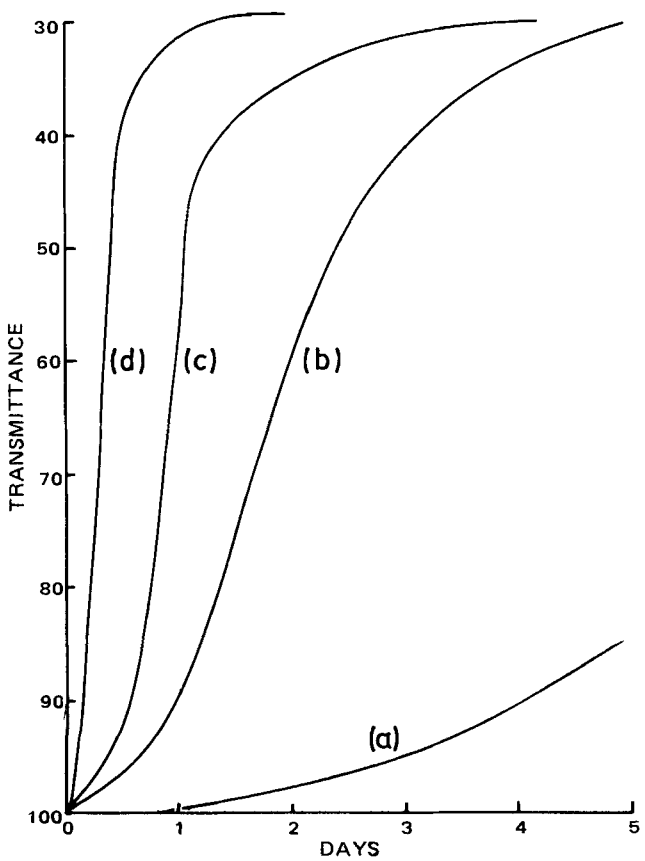


Figure 3—*Effect of temperature on the development of the 15,200-cm^{-1} (658-nm) peak of the* p*-benzoquinone–I complex in pH 7 phosphate buffer. Key: a, 25°; b, 35°; c, 45°; and d, 55°.*

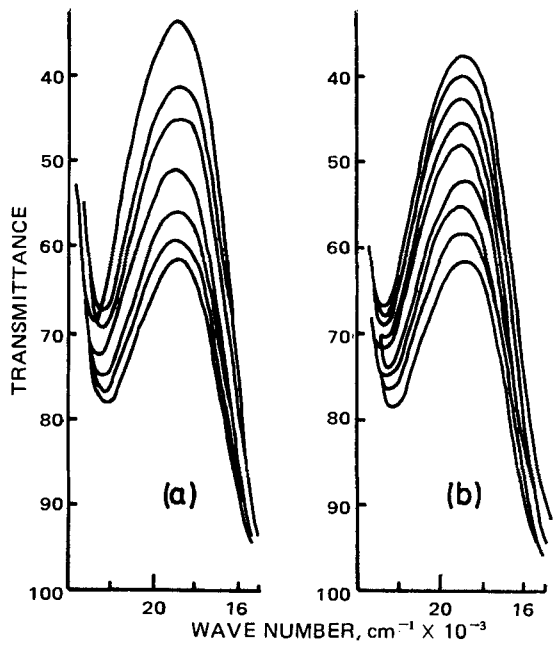


Figure 4—*Effect of I (2 × 10^{-2} M) on the absorption band at 18,800 cm^{-1} (532 nm) ascribed to the* p*-benzoquinone (2 × 10^{-4} M)–proline (7 × 10^{-2} M) complex in pH 7 phosphate buffer at 55°. Key: a, peak decreasing with time (0, 7, 10, 15, 20, 25, and 35 min); and b, peak increasing with time (35, 50, 60, 70, 85, 100, 120, 150, and 180 min).*

Interaction of I and Proline with *p*-Benzoquinone—To clarify the interactions of this termolecular system, three separate sets of experiments were performed. In Experiment A, both proline and I were added more or less simultaneously to the quinone. Experiment B concerned the effect of adding proline to a solution to which I had been added to the quinone several hours previously. Experiment C examined the effect of adding I to a solution in which a proline–quinone complex equilibrium had been established.

Experiment A—The addition of proline and I to the *p*-benzoquinone solution caused changes in absorption that were identical to those caused when proline alone was added to the quinone (4). Compound I can be dissolved in *p*-benzoquinone for up to 4 hr before adding proline without affecting the rate of formation of the proline–quinone complex.

Experiment B—When I was added to *p*-benzoquinone and allowed to stand for many hours, a band at 15,200 cm^{-1} (658 nm) gradually formed. The addition of proline to the solution in which this band was fully formed had no effect whatsoever. Old solutions of proline and quinone also gave rise to a similar band, preceded by the formation of a proline–quinone complex (4).

Experiment C—The addition of proline to *p*-benzoquinone resulted in the formation of a reversible complex with maximum absorption at 18,800 cm^{-1} (532 nm). After many hours, this absorption slowly decreased and was replaced by an absorption at 15,200 cm^{-1} (658 nm), belonging to some later formed reaction product (4). The addition of I to a solution

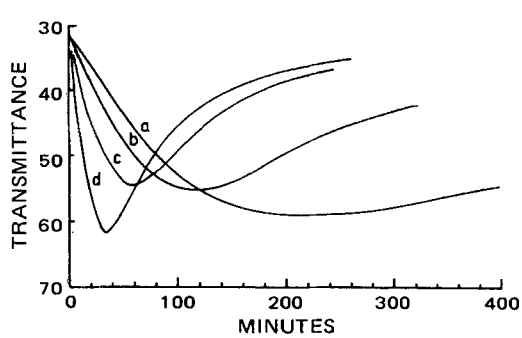


Figure 5—*Effect of varying concentration of I on changes in the proline–*p*-benzoquinone complex as measured by the absorption band at 18,800 cm^{-1} (532 nm) in phosphate buffer at pH 7 and 55°. Key: a, 10^{-3} M; b, 4 × 10^{-3} M; c, 10^{-2} M; and d, 2 × 10^{-2} M.*

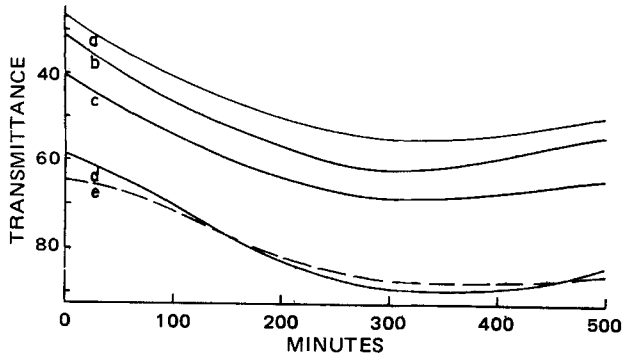


Figure 6—*Effect of I (2×10^{-2} M) on the absorption band at 18,800 cm^{-1} (532 nm) formed by mixing, at pH 7 and 25°, 2×10^{-4} M p-benzoquinone and the following concentrations of proline: (a) 4×10^{-1} M, (b) 7×10^{-2} M, (c) 1.5×10^{-2} M, (d) 2×10^{-4} M, and (e) 10^{-4} M.*

of *p*-benzoquinone and proline in which the complex absorbing at 18,800 cm^{-1} (532 nm) was fully formed but had not begun to decompose to give the absorption at 15,200 cm^{-1} (658 nm) gave some surprising changes. There was an initial slow decrease in the absorption at 18,800 cm^{-1} (532 nm) followed by an increase in the absorbance back toward the initial absorbance (Fig. 4).

Figures 5 and 6 show the effect of varying the drug and proline concentrations. As the drug concentration increased, so did the rate of its interaction with the proline–quinone system. From Fig. 6 it can be deduced that the concentration of proline does not affect the initial interaction rate—only the rate at which the absorption peak regains its equilibrium value.

Figure 7 shows the effect of temperature on the interaction of the drug on the quinone–proline system. As expected, raising the temperature caused an increase in the rate of interaction for both the decrease and later increase of the complex. The higher the temperature above 35°, the greater was the effect of temperature on the decrease of the complex as compared with the later increase. This trend was reversed below 35°. A similar effect was noted later with the histamine–quinone system.

From these experiments, it can be concluded that:

1. Compound I does not interfere with the initial complexing reaction between proline and the quinone.

2. There is a very slow chemical interaction between I and quinone with a large induction time, showing that intermediates are produced before the final product absorbing at 15,200 cm^{-1} (658 nm). This final product may be a disubstituted quinone (9).

3. A reaction between I and the proline–quinone complex leads to a decrease in the concentration of the complex.

Presumably there is competition for complexing with the quinone between I and proline. However, at a later time, the concentration of the complex returns to its original value with proline alone due to either an increase in the proline available for complexing or a decrease in drug. The proline exists mainly in its zwitterionic form at pH 7, but interaction is *via* the neutral amino form. At these pH's, this form is present only in about 1 part in 1000 (4). Similarly, the drug exists mainly in its ionized form at pH 7 (1), although some unionized form is present. This system

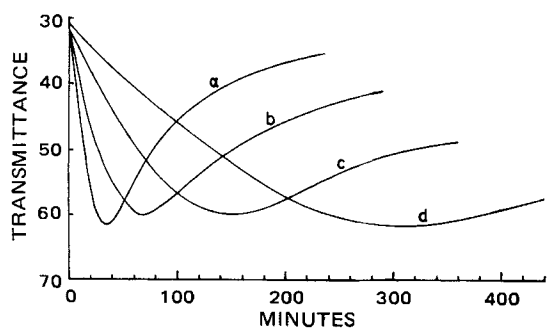


Figure 7—*Effect of I (2×10^{-2} M) on the absorption band at 18,800 cm^{-1} (532 nm) formed by mixing proline (7×10^{-2} M) with p-benzoquinone (2×10^{-4} M) at 25° in pH 7 phosphate buffer. Key: a, 55°; b, 45°; c, 35°; and d, 25°.*

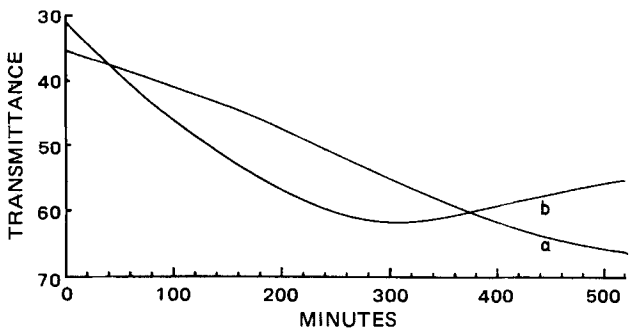


Figure 8—*Effect of I (2×10^{-2} M) on the absorption at 18,800 cm^{-1} (532 nm) of the proline (7×10^{-2} M)–p-benzoquinone (2×10^{-4} M) complex in phosphate buffer at 25°. Key: a, pH 8.0; and b, pH 7.0.*

is obviously very complicated; the various equilibria producing complexing forms of proline and drug are interdependent. Thus, either the shift of the equilibrium to produce more unionized proline or to produce less unionized drug would explain the results, the latter seeming more suitable.

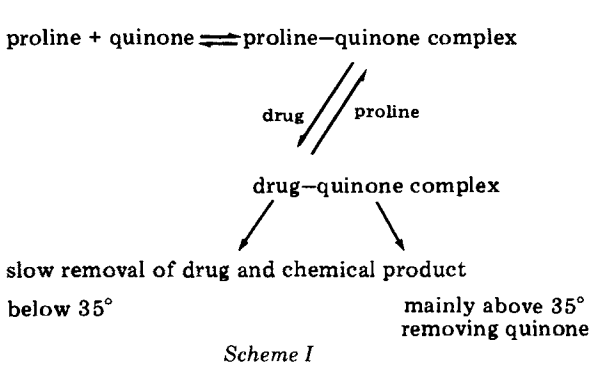
Another explanation for the slow recovery of the proline–quinone complex concentration is the removal of drug by dimerization, although there is not any evidence for this, or by some other chemical reaction. However, at this stage of the interaction, the quinone is not removed from solution, as seen by the return of the complex absorption to its initial value at temperatures less than 35°. Only at higher temperatures is quinone removed from the solution and the complex does not regain its initial value with time. These various reactions are summarized in Scheme I.

It was previously observed that an increase in pH leads to a considerable apparent increase in the reaction rate between proline and *p*-benzoquinone (4). Therefore, the interaction of the drug with the proline–quinone system at pH 8 also was investigated (Fig. 8). There was the decrease of the band associated with the complex but no subsequent increase as at pH 7. The binding between the drug and *p*-benzoquinone at pH 8 apparently is stronger than that between proline and *p*-benzoquinone. Thus, pH is a strong determining factor in the interaction of this termolecular system. The effect of the drug on the hydroxyproline–quinone system is similar to that described for proline, but the reaction rates are slightly different.

Figure 9 shows the effect of some related drugs (II–IV) on the proline–quinone complex. Compound IV, a triazolopyrimidine, had only a marginal effect; the other drugs, all triazolopyrazines, had a marked effect, albeit in different ways. Among the triazolopyrazines, only I exhibited the reversal in the downward trend of the complex concentration at pH 7. None of these drugs other than I showed any spectroscopic evidence of an interaction with *p*-benzoquinone alone, although II and III obviously interacted with the proline–*p*-benzoquinone system (Fig. 9).

There is now a body of evidence showing that amines and amides, especially biological amines and amides in the unionized form, complex with quinones and other charge acceptors (3, 4, 8). If the drugs interact in a similar manner, then this interaction explains the rather slow forward reaction times; at the pH values used, the amines or amides are mainly in the ionized XNH_3^+ or XNH_2^+ form, so the effective concentration of XNH_2 or XNH is quite low.

Interaction of I with Histamine–*p*-Benzoquinone Complex—In the presence of I, there was a change in both the apparent rate constants



Scheme I

Table I—Apparent and Corrected Rate Constants for the Interactions of Histamine with *p*-Benzoquinone in the Presence of I in pH 7 Phosphate Buffer[a]

k_1, M^{-1} min^{-1}	k_2, min^{-1}	k_3, min^{-1}	$\frac{k_1}{k_2}$, M^{-1}	Temperature
1.51×10^{-2} (8.4)	4.53×10^{-4}	2.72×10^{-6}	33.3 (18,700)	25°
4.37×10^{-2} (1.26×10)	3.52×10^{-3}	6.77×10^{-5}	12.4 (3570)	35°
1.19×10^{-1} (2.07×10)	2.63×10^{-2}	1.21×10^{-3}	4.5 (784)	45°
2.87×10^{-1} (2.92×10)	1.02×10^{-1}	7.57×10^{-3}	2.8 (286)	55°

[a] Data in parentheses are corrected for histamine concentration only and not for I.

and the thermodynamic parameters of the histamine–quinone complex (Tables I and II). The effect of the drug was greater the longer it was dissolved in quinone solution prior to the addition of histamine, confirming that the drug interacts slowly with the quinone, forming a chemical compound. After 24 hr, almost all quinone had interacted with the drug, as evidenced by the negligible reaction with histamine if added after this time. On the other hand, there was no evidence of the 15,200 cm^{-1} (658 nm) peak at this time.

The data in Table I can be compared with the data for the interaction of histamine with *p*-benzoquinone alone given in Tables 1 and 2 of Ref. 3[2]. The data in Table I are compatible with a competitive reaction between the drug and the histamine for complexing with *p*-benzoquinone. There was a decrease in the apparent interaction rate between histamine and the *p*-benzoquinone in the presence of the drug at lower temperatures. However, at higher temperatures, there was an apparent increase in the interaction rate of histamine, showing that a more complicated set of interactions occurred in the presence of the drug at these elevated temperatures. A similar effect of temperature was already noticed for the interaction of the drug with the proline–*p*-benzoquinone system (4). Presumably, at the higher temperatures, the chemical interactions become significantly large compared to the complexing mechanism that predominates at the lower temperatures.

Obviously, any rate constants derived for the interaction of the drug with the *p*-benzoquinone from these termolecular systems will contain a contribution from the irreversible chemical interactions. The apparent rate constants for the drug–quinone complex derived from these data, assuming a simple competition between the drug and histamine, are:

$$k_1 = 1.04 \times 10^{-2} \text{ liter mole}^{-1} \text{ min}^{-1} \quad \text{(Eq. 1)}$$

$$k_2 = 7.28 \times 10^{-4} \text{ min}^{-1} \quad \text{(Eq. 2)}$$

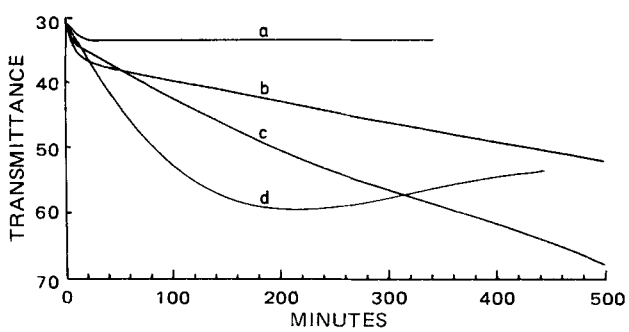


Figure 9—*Effect of different drugs (10^{-3} M) on the absorption at 18,800 cm^{-1} (532 nm) of the proline (7×10^{-2} M)-p-benzoquinone (2×10^{-4} M) complex in pH 7 phosphate buffer at 25°. Key: a, IV; b, III; c, II; and d, I.*

[2] Due to a mistake in the computer printout, all values of k_1, k_2, and k_3 in Ref. 3 should be multiplied by 10^5. Other parameters are not affected.

Table II—Apparent and Corrected Thermodynamic Data for the Interaction of Histamine with *p*-Benzoquinone in the Presence of I in pH 7 Phosphate Buffer[a]

	ΔH	ΔS	ΔG	E_A
k_1	18.3 (6.6)	−36.6 (−31.2)	29.2 (15.9)	18.9 (7.2)
k_2	34.6	11.4	31.2	35.2
k_3	50.9	56	34.2	51.5
$\frac{k_1}{k_2}$	−16.3 (−28)	−48.0 (42.6)	−2.0 (15.3)	

[a] Data in parentheses are corrected for histamine concentration only and not for I. H = enthalpy, S = entropy, G = free energy, and E_A = activation energy; all parameters are in kilocalories mole^{-1} except entropy which is in entropy units.

with $k_1/k_2 = 14.3\ M^{-1}$, and:

$$k_3 = 2.05 \times 10^{-5} \text{ min}^{-1} \quad \text{(Eq. 3)}$$

all at 25°. Since the ionization constant of the drug is unknown, it is not possible to give corrected data. The thermodynamic data in Table II are averaged out over the temperature range and again must contain substantial contributions from the irreversible chemical interactions.

CONCLUSIONS

The addition of certain heterocyclic nitrogen compounds to solutions of the electron-acceptor *p*-benzoquinone causes no change in the absorption spectrum in the near UV and visible regions. The only exception is I, which shows very slow changes after a long induction period. However, the addition of these compounds to solutions containing electron donors and acceptors has marked spectral effects which demonstrate that these compounds do initially complex with electron acceptors and later form chemical compounds with them. At temperatures below 35°, the interaction is due to some form of complexing; at higher temperatures, irreversible chemical reactions between the drugs and acceptors occur quite rapidly. This study illustrates the utility of using termolecular systems to characterize reactions that cannot be characterized by observing directly the interactions between the compounds and the acceptors. The pH effects suggest that these amines and amides interact in their neutral form—not the ionic form that predominates at the pH's studied.

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