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[x] To whom inquiries should be directed.

Equilibrium Reaction of Pyrazolodiazepinones in Aqueous Solution

W. H. HONG, C. JOHNSTON, and D. SZULCZEWSKI [x]

Abstract □ This study of the behavior of some pyrazolodiazepinones in aqueous solution at near ambient temperature indicated that they form stable equilibrium mixtures consisting of ring and opened forms. Under isothermal conditions, mixtures are produced whose composition is dependent on pH and may vary from that corresponding to essentially complete ring opening to complete closure. Ring closure equilibrium constants were calculated, and the influence of methyl substitution was determined. Substitution of a methyl group for an amido hydrogen in the open form results in a fivefold increase in this constant. Methylation of the terminal amino group, however, did not cause a corresponding increase and may not significantly affect equilibrium.

Keyphrases □ Pyrazolodiazepinones, various—equilibrium reaction in aqueous solution, effect of pH and methyl substitution □ Equilibrium—various pyrazolodiazepinones in aqueous solution, effect of pH and methyl substitution □ Structure–activity relationships—various pyrazolodiazepinones, equilibrium reaction in aqueous solution, effect of pH and methyl substitution

1-Ethyl - 4,6 - dihydro-3-methyl-8-phenylpyrazolo[4,3-*e*][1,4]diazepin-5(1*H*)-one (I*a*) (ripazepam) is an investigational new drug of the diazepinone class being evaluated for clinical utility (1–3). This pyrazolodiazepinone, in contrast to 1,4-benzodiazepinones, is easily hydrolyzed to yield the open chain compound 4-(2-aminoacetamido)-5-benzoyl-1-ethyl-3-methylpyrazole (II*a*) (1).

Reports concerning hydrolysis or stability of benzodiazepinones (4–8) indicate that the open form in this series is usually unstable under the conditions required to hydrolyze the diazepinone ring, so it does not permanently accumulate. In addition to the ease of hydrolysis, other characteristics associated with the reaction of the pyrazolodiazepinones in aqueous solution are sufficiently different from those of the benzodiazepinones to warrant further study.

EXPERIMENTAL

Reagents—All chemicals used to prepare 0.1 and 0.05 *M* acetate, citrate, phosphate, and borate buffers of known pH values were reagent grade and were used without further purification. Hydrochloric acid solutions were prepared from prestandardized volumetric solutions[1]. The solvents for equilibrium studies, including hydrochloric acid solutions and various buffers, were adjusted to an ionic strength of 0.3 with potassium chloride.

Compound I*a* was from an experimental batch[2] with a purity of 99.75% *via* differential scanning calorimetric analysis. 4-(2-Aminoacetamido)-5-benzoyl-1-ethyl-3-methylpyrazole (II*a*) dihydrochloride and 1-ethyl-4,6- dihydro -3,4- dimethyl-8-phenylpyrazolo[4,3-*e*][1,4]diazepin-5(1*H*)-one (V*a*) were used as received[3].

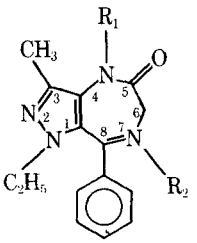
Synthesis of 4-[2-(Methylamino)acetamido]-5-benzoyl-1-ethyl-3-methylpyrazole Hydrochloride (VII*c*)—To a solution of I*a*, 10 g in 100 ml of dichloromethane, 8 g of methyl fluorosulfonate was added dropwise over 5 min with stirring. The reaction mixture was allowed to stir for another 2 hr and was then poured into 1 liter of ether with stirring. The yellow precipitate was collected, rinsed with ether, and dissolved in 160 ml of water. The resulting solution was washed with 3 × 30-ml portions of chloroform, and the washings were discarded. The aqueous layer was adjusted to pH 8 to obtain optimal turbidity with sodium hydroxide solution and subsequently extracted with 5 × 30-ml portions of chloroform.

The combined chloroform extract was dried over anhydrous sodium sulfate and filtered. Then dried hydrochloric acid gas was introduced into the chloroform solution until the turbidity was no longer increased. The solvent was removed *in vacuo* using a flash evaporator. The residue thus obtained was recrystallized several times from acetonitrile to afford fine white needles, mp 187–188° dec., in a yield of 6.8 g (54%); UV (methanol): $\epsilon_{258.5} = 1.23 \times 10^4$.

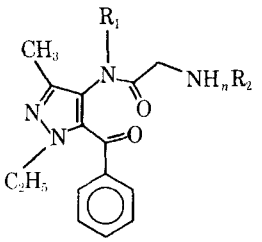
Anal.—Calc. for $C_{16}H_{21}ClN_4O_2$: C, 57.05; H, 6.28; Cl, 10.53; N, 16.64. Found: C, 57.27; H, 6.21; Cl, 10.57; N, 16.65.

Determination of Methylation Site in VII*a*—One gram of VIIc in

[1] Acculute standard volumetric solution, Anachemia Chemicals Ltd.
[2] RxX Lot 41201.
[3] Dr. H. DeWald, Chemistry Department, Parke, Davis & Co.



Ia: R_1 = H, no R_2
Ib: R_1 = R_2 = H, charge = + 1
Va: R_1 = CH_3, no R_2
Vb: R_1 = CH_3, R_2 = H, charge = +1
VIII: R_1 = H, R_2 = CH_3, charge = +1
IX: R_1 = R_2 = CH_3, charge = +1



IIa: R_1 = R_2 = H, n = 1
IIb: R_1 = R_2 = H, n = 2, charge = +1
IIc: dihydrochloride
VIa: R_1 = CH_3, R_2 = H, n = 1
VIb: R_1 = CH_3, R_2 = H, n = 2, charge = +1
VIIa: R_1 = H, R_2 = CH_3, n = 1
VIIb: R_1 = H, R_2 = CH_3, n = 2, charge = +1
VIIc: hydrochloride

25 ml of 0.4 *N* HCl was sealed in a 50-ml glass ampul and heated in a steam bath for 3 days. After cooling, the hydrolysis mixture was quantitatively transferred into a 250-ml separator with 20 ml of water. The resulting yellow solution was washed with 3 × 30-ml portions of chloroform, and the washings were discarded. The aqueous layer was then made alkaline (pH >12) and washed with 3 × 30-ml portions of chloroform, and the washings were discarded again. The aqueous layer was neutralized with 2 *N* HCl and evaporated to dryness *in vacuo* at 35° using a flash evaporator.

The white solid thus obtained was repetitively treated with hot methanol, yielding an off-white powder. This compound showed only one TLC spot, which had the same characteristics as those of sarcosine when the sample was spotted on a silica gel plate[4], developed in 2-propanol–water (70:30) and 2-propanol–concentrated ammonia (70:30), and detected by ninhydrin reagent.

Synthesis of 1-Ethyl-1,4,5,6-tetrahydro-3,4,7-trimethyl-5-oxo-8-phenylpyrazolo[4,3-*e*][1,4]diazepinium Iodide (IX)—Compound IX was prepared as follows. To a solution of Va (0.5 g in 4 ml of acetonitrile) in a 50-ml glass ampul, a solution of methyl iodide (0.31 g in 1 ml of acetonitrile) was added dropwise over 2 min with shaking. The ampul was then sealed and heated in a steam bath for 3 hr. After cooling, the mixture was poured into 500 ml of ether with stirring. The yellow precipitate was filtered, rinsed with ether, and recrystallized from the acetonitrile–ether mixture, giving a yellow crystalline powder, mp 230° dec., in a yield of 0.59 g (79%); UV (acetonitrile): $\epsilon_{381} = 4.50 \times 10^3$ and $\epsilon_{301} = 1.12 \times 10^4$.

Anal.—Calc. for $C_{17}H_{21}IN_4O$: C, 48.12; H, 4.99; I, 29.91; N, 13.21. Found: C, 47.92; H, 4.79; I, 29.50; N, 13.08.

Determination of Methylation Site in IX—Compound IX, 0.45 g, in 20 ml of 0.4 *N* HCl was sealed in a 50-ml glass ampul and heated in a steam bath for 2 days. After cooling, the hydrolysis mixture was filtered, and the yellow filtrate was quantitatively transferred into a 60-ml separator with 10 ml of water. The yellow solution was washed with 3 × 20-ml portions of chloroform, and the washings were discarded. The aqueous layer was then made alkaline (pH >12) and washed with 3 × 20-ml portions of chloroform, and the washings were discarded again. The aqueous layer was neutralized with 2 *N* HCl and evaporated to dryness *in vacuo* at 35° using a flash evaporator.

[4] Q1-F, Quantum Industries.

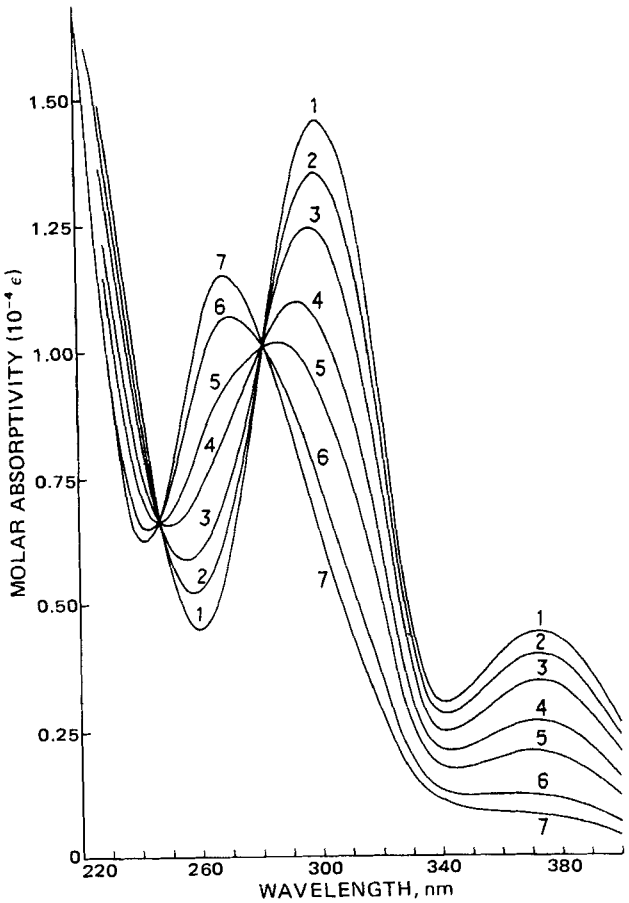


Figure 1—*Sequential UV spectra in 1.0* N *HCl accompanying equilibration of Ia. Reaction solution was 0.1 M citrate buffer, pH 4.0, μ = 0.3. Spectra were obtained at the following times: 1, 2 min; 2, 10 min; 3, 20 min; 4, 40 min; 5, 1 hr; 6, 2 hr; and 7, 72 hr.*

The yellowish-white solid thus obtained was repetitively treated with hot methanol, yielding an off-white powder. This compound showed one TLC spot, which had the same chromatographic characteristics as those of sarcosine when the sample was spotted on a silica gel plate[4], developed in 2-propanol–water (70:30) and 2-propanol–concentrated ammonia (70:30), and detected by ninhydrin reagent.

Instrumentation—The pH measurements were made using direct reading, digital pH meters[5,6]. UV spectra were obtained using a recording UV spectrophotometer[7].

pKa Determination of IIa—Portions, 107 mg, of IIa dihydrochloride were accurately weighed and quantitatively transferred to each of 10 20-ml beakers. Distilled water, 15 ml, was added to a beaker, the compound was allowed to dissolve, and a known volume of 0.5 *N* NaOH (0–1.60 ml in 0.2-ml increments) was added. The pH of the resulting solution was recorded immediately after preparation (2 min) and periodically thereafter for 7 min. This process was then repeated for each different volume of 0.5 *N* NaOH added.

The initial pH was determined by extrapolation of the pH *versus* time curves obtained to zero time. A plot was then made of initial pH *versus* alkali added, and the pKa was calculated as described by Albert and Serjeant (9).

Spectrophotometric pKa Determination of Ia and Va—A stock solution of Ia or Va in water, 1.8×10^{-3} *M*, containing 10% methanol was freshly prepared. Aliquots of 2 ml were diluted to 25 ml with hydrochloric acid solution, 0.01 *M* acetate buffer, and 0.01 *M* phosphate buffer, separately, to cover the pH range of 1.3–7.3. The resulting solutions were then scanned from 400 to 220 nm *versus* the respective solvents. The pKa's were then calculated from the absorbances at 300 nm as described by Albert and Serjeant (10).

Equilibrium Studies—Approximately 0.08 mEq of Ia or IIc was ac-

[5] Model 701, Orion digital pH meter.
[6] Model DR, Sargent digital pH meter.
[7] Cary model 11 or 14.

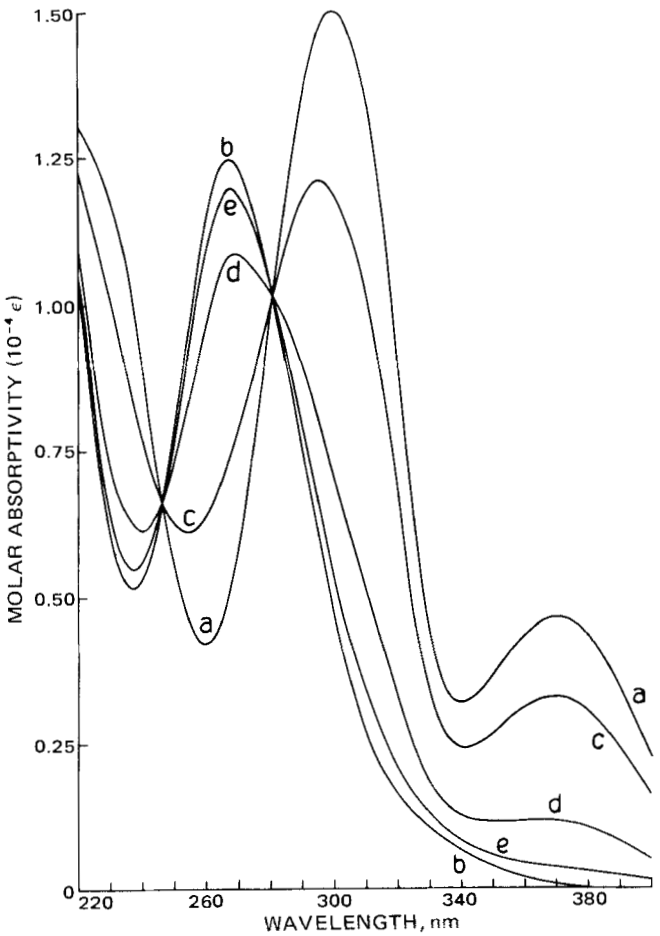


Figure 2—*UV spectra in 1.0* N *HCl of* Ia (*a*), *IIa* (*b*), *and equilibrium mixtures as produced at pH 5.0* (*c*), *4.0* (*d*), *and 2.0* (*e*).

curately weighed and quantitatively transferred into a 50-ml volumetric flask. Then 1 ml of methanol was added to dissolve the compound. The reaction was initiated by bringing to volume with an adequate solvent or buffer of known pH and constant ionic strength (27°, $\mu = 0.3$). At appropriate intervals, a 2-ml aliquot was withdrawn and diluted to 25 ml with 1 *N* HCl. The resulting solution was immediately scanned from 400 to 220 nm *versus* 1 *N* HCl using 1-cm silica cells. Spectra were obtained until they became redundant. Hydrochloric acid (1.0 *N*) was used as the solvent for spectral measurement to avoid changes in spectra resulting from varying degrees of protonation and because it effectively quenched the reaction.

Determination of Composition of Equilibrium Mixtures—The composition of equilibrium mixtures was calculated from the redundant spectra as follows:

$$\%\,\mathrm{I} = \frac{a_{\lambda 371}}{4.76 \times 10^3\, C} \frac{1}{} 100 \qquad \text{(Eq. 1)}$$

$$\%\,\mathrm{II} = 100 - \%\,\mathrm{I} \qquad \text{(Eq. 2)}$$

where $a_{\lambda 371}$ is the absorbance of the final dilution of the reaction solution, C is the initial molar concentration of Ia or IIc, 4.76×10^3 is the molar absorptivity of Ia in 1 *N* HCl at 371 nm, [I] = [I*a*] + [I*b*], and [II] = [II*a*] + [II*b*].

RESULTS AND DISCUSSION

A preliminary study indicated that the UV spectrum of Ia, as determined in aqueous buffers having a pH of 6 or less, changed quite rapidly. Although the rate at which the spectrum changed depended on the pH in a complex manner, the difference between initial and final spectra was regular and became greater as the pH diminished.

Sequential spectra were obtained from reaction solutions of Ia maintained at 27° in buffers of known but varying pH (Fig. 1). Absorption at 371 and 300 nm decreased whereas absorption at 266 nm increased concurrently until the spectra became redundant with time. The shape of the redundant spectra varied with the pH of the reaction solution (Fig. 2).

Table I—Composition of Equilibrium Mixtures (27°)

Equilibration of Ia		Equilibration of Va	
pH	f_{II}[a]	pH	f_{VI}
1.18	0.95	1.44	0.71
2.10	0.95		
3.11	0.92	2.24	0.69
3.76	0.89	3.04	0.62
4.19	0.83	3.98	0.34
4.80	0.55	5.05	0.07
5.40	0.23		
5.80	0.12		
6.35	0.06		
7.20	0.02		

[a] Results at pH 1.18 and 2.10 were obtained by polarographic analysis. All other results were obtained spectrophotometrically.

Solutions of Ia at pH 1–6 were prepared and allowed to equilibrate until spectral redundancy was attained. The remaining portion of these solutions, after adjusting to pH 8.0, generated the spectrum of Ia. These results, together with information from previous studies (1), indicate that Ia undergoes a reversible reaction involving conversion of Ia to equilibrium mixtures containing I and II.

Additional evidence in this regard was obtained by studying the behavior of IIc after exposure to the same conditions used to study Ia. As seen from Fig. 2, the spectrum of IIc closely resembled the terminal spectra obtained when Ia was allowed to equilibrate at pH 2.5 or less. Some difference, however, existed between these spectra. The terminal spectra had greater absorption in the 371-nm region and somewhat less absorption at the 266-nm maximum of IIa than would be expected if Ia were completely converted to II. Furthermore, when IIc was allowed to equilibrate at pH 2.5–1.0, its spectrum changed slightly, and spectra identical to those obtained by equilibrating Ia at the same acidic pH values were produced.

Reaction solutions of IIc having pH 2.5 or greater produced redundant spectra which differed from those of IIa to an increasing degree as the pH increased. At pH 7.0 or higher, spectra consistent with equimolar conversion of IIa to Ia were obtained. The same set of isosbestic points existed in the sets of spectra obtained from equilibration starting with

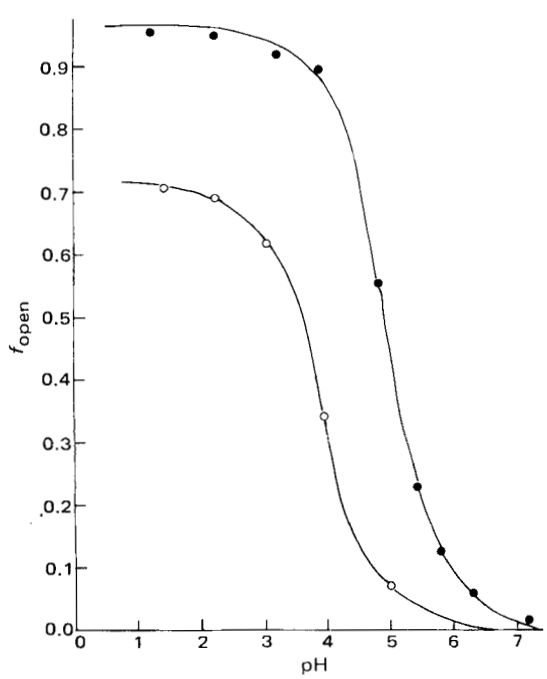


Figure 3—*Plot of the composition of equilibrium mixtures* versus *pH* (27°). *Key:* ●, *experimentally determined values for the I–II system; and* ○, *experimentally determined values for the V–VI system. The solid lines are values calculated using Eq. 5 with* $K^*_{II} = 1.13 \times 10^6\ M^{-1}$ *and* $K^*_{V\text{-}VI} = 6.1 \times 10^6\ M^{-1}$.

Ia (Fig. 1) as in the sets of spectra obtained from equilibration starting with IIc.

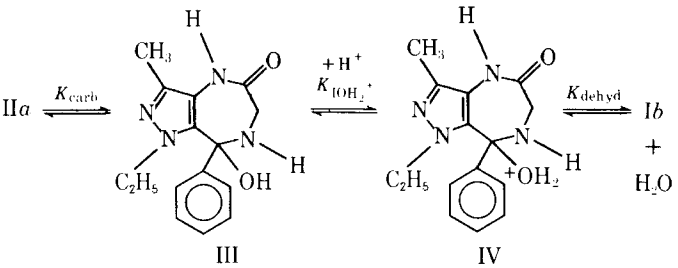
The shape of the redundant spectra produced upon equilibration was pH dependent. Since UV properties of both Ia and IIa are known, the composition of equilibrium mixtures corresponding to these spectra can be calculated. Analysis of the redundant spectra produced at various pH values gave the results in Table I and Fig. 3. The fraction of total open and closed species present as open species at equilibrium, f_{II} (see *Appendix*), increased in a sigmoidal fashion as pH decreased until, at pH values less than approximately 2.5, it became independent of pH but was not unity.

Results obtained thus far may be explained on the basis of Scheme I, representing the simultaneous occurrence of three equilibrium reactions.

$$IIb \overset{K_{a2}^{IIb}}{\rightleftharpoons} IIa + H^+ \overset{K^*}{\rightleftharpoons} Ib + H_2O \overset{K_{a1}^{Ib}}{\rightleftharpoons} Ia + H^+$$

Scheme I

The reaction accommodates intermediate carbinolamine formation from the free base form of IIa and the protonated form of Ia (Ib) in accord with established mechanistic principles (11). The reaction is probably more complicated than is indicated by Scheme I and likely involves (11) the equilibria shown in Scheme II.



Scheme II

Intermediate compounds (III and IV), however, were never present in detectable concentrations. The sets of isosbestic points observed in the complete conversion of II to I or virtually complete conversion of I to II (Fig. 1) were the same and explicable on the basis of a binary system consisting of only I and II. Furthermore, the spectra of Ia in less reactive nonaqueous solvents such as acidified acetonitrile or methanol emulated those initially observed in water (Table II).

The net reaction resulting from the three reactions involving carbinolamine formation and dehydration is given as Scheme III.

$$IIa + H^+ \overset{K^*}{\rightleftharpoons} Ib + H_2O$$

Scheme III

The ring closure equilibrium constant, $K^{*\prime}$, is then defined by:

$$K^* = \frac{[Ib][H_2O]}{[IIa][H^+]} \qquad \text{(Eq. 3)}$$

$$K^{*\prime} = \frac{[Ib]}{[IIa][H^+]} \qquad \text{(Eq. 4)}$$

The equilibrium constant, $K^{*\prime}$, which governs ring closure is actually a composite, being equal to the product $K_{carb}K_{IOH_2}K_{dehyd}$.

Consideration of the equilibria involved in Scheme I leads to the following expression[8], which relates the composition of equilibrium mixtures to the hydrogen-ion concentration:

$$f_{II} = \frac{K_{a2}^{IIb} + [H^+]}{K_{a2}^{IIb} + [H^+] + K_{a2}^{IIb}K^{*\prime}\{K_{a1}^{Ib} + [H^+]\}} \qquad \text{(Eq. 5)}$$

The limiting forms of Eq. 5 are:

$$\lim f_{II}^{H^+ \to \infty} = \frac{1}{1 + K_{a2}^{IIb}K^{*\prime}} \qquad \text{(Eq. 6)}$$

$$\lim f_{II}^{H^+ \to 0} = \frac{1}{1 + K_{a1}^{Ib}K^{*\prime}} \qquad \text{(Eq. 7)}$$

Equations 6 and 7 are consistent with results obtained thus far in that they indicate that two regions of pH independence can exist, that the limiting values of f_{II} need not be unity, and that the limiting value of f_{II} reached at more acidic pH values should be greater than the limiting value reached at less acidic pH's[9]. Since Eq. 5 is in qualitative agreement with results obtained, it will be accepted as valid and tested further.

The value of $K^{*\prime}$, *i.e.*, the net equilibrium constant for the equilibrium reaction between open and closed forms, can be calculated from Eq. 5 and the experimental results. At pH 5.5 or less, the hydrogen-ion concentration is much greater than the value of K_{a2}^{IIb}, so Eq. 5 reduces to:

$$\frac{1}{f_{II}} = (1 + K^{*\prime}K_{a2}^{IIb}) + K^{*\prime}K_{a2}^{IIb}K_{a1}^{Ib}\frac{1}{[H^+]} \qquad \text{(Eq. 8)}$$

Equation 8 indicates that a plot of $1/f_{II}$ *versus* $1/[H^+]$ should be a straight line whose slope is $K^{*\prime}K_{a2}^{IIb}K_{a1}^{Ib}$. Since the two ionization constants are known, $K^{*\prime}$ can be calculated (Fig. 4). This method of calculating $K^{*\prime}$ uses data obtained at pH values at which the spectral measurement of f_{II} is most accurate and avoids the analytical difficulty encountered when the redundant spectra, representing equilibration, are similar to those of IIa.

When the value of $K^{*\prime}$ (= 1.13×10^6) as previously determined is substituted in Eq. 5, together with corresponding dissociation constants (*Appendix*) and values of f_{II} calculated by mathematically changing the hydrogen-ion concentration, the curve shown as the solid line in Fig. 3 is generated. As seen, this curve closely approximates experimentally determined results[10].

The preceding considerations developed for the II–I system are general and can be applied to other diazepinone systems to interpret results and to compare corresponding equilibria. The utility of this approach to determine the influence of structural modification on the ring closure equilibrium constant, $K^{*\prime}$, is illustrated by the following examples.

The 4-*N*-methyl derivative of Ia, Va, behaves as does Ia in kind but not degree (Fig. 3 and Table I). The values of f_{VI}, when plotted *versus* pH, "break" at a lower pH, and the limiting value of f_{VI} attained at lower pH values is less than that for the system involving II. Since the pKa of the open form of Va, VIa, is similar to that of IIa, Eqs. 9 and 10 are valid:

$$K^{*\prime}\,(\text{VI–V system}) = \left\{\frac{1 - \lim f_{VI}^{H^+ \to \infty}}{\lim f_{VI}^{H^+ \to \infty}} \cdot \frac{\lim f_{II}^{H^+ \to \infty}}{1 - \lim f_{II}^{H^+ \to \infty}}\right\} K^{*\prime}\,(\text{I–II system}) \qquad \text{(Eq. 9)}$$

$$K^{*\prime}\,(\text{VI–V system}) = 5.4K^{*\prime}\,(\text{II–I system}) \qquad \text{(Eq. 10)}$$

This result indicates that substitution of a methyl group for an amido hydrogen results in approximately a fivefold increase in the value of the equilibrium constant governing ring formation.

Table II—UV Spectral Properties of Various Compounds or Ions

Compound or Ion	Solvent	λ_{max} (log ϵ)
Ia	0.1 *M*, pH 7 phosphate buffer (aqueous)	305 s (3.55)
Ib	0.1 *N* HCl (aqueous)	300 (4.18), 371 (3.67)
Ib	1.0 *N* HCl (aqueous)	300 (4.18), 371 (3.67)
Ib	Methanol, 0.1 *N* H_2SO_4	298 (4.14), 377 (3.67)
Ib	98% Acetonitrile–2% 5 *N* HCl (aqueous)	300 (4.17), 377 (3.68)
I^-	0.1 *N* NaOH (aqueous)	346 (3.62)
IIb	0.01 *N* HCl (aqueous)	266 (3.997)
IIb	0.1 *N* HCl (aqueous)	266 (3.996)
IIb	1.0 *N* HCl (aqueous)	266 (3.998)
IIb	2.0 *N* HCl (aqueous)	269 (3.980)
IIb	5.0 *N* HCl (aqueous)	272 (3.979)
Va	0.1 *M*, pH 7 phosphate buffer (aqueous)	251 s (4.04), 305 s (3.52)
Vb	0.1 *N* HCl (aqueous)	300 (4.17), 373 (3.66)
Vb	1.0 *N* HCl (aqueous)	300 (4.14), 372 (3.64)
VIIb	0.1 *N* HCl (aqueous)	266 (4.001)
IX	98% Acetonitrile–2% water	301 (4.05), 381 (3.65)

[8] See *Appendix* for derivation and definitions.

[9] Some caution must be exercised in applying this conclusion since if both $K_a^{open}K^{*\prime}$ and $K_a^{closed}K^{*\prime}$ are either very large or very small as compared to unity, there would be no observable difference between the limits as attained at more or less acidic pH values.

[10] Polarographic analysis of the composition of equilibrium mixtures produced at more acidic pH values indicate $\lim f_{II}^{H^+ \to \infty} = 0.95$ rather than 0.93 as determined by UV measurement.

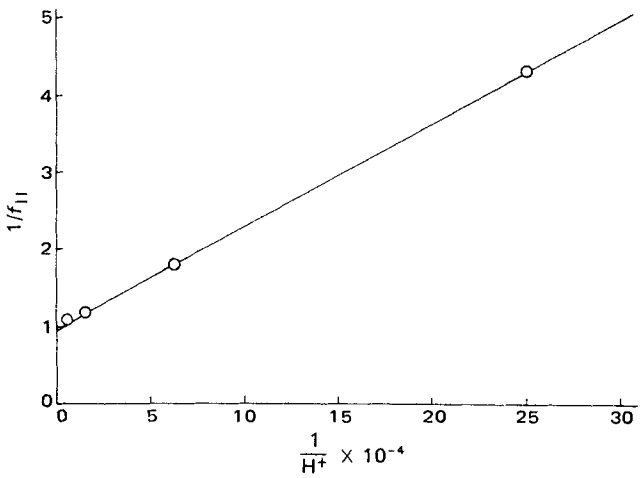


Figure 4—*Plot of* $1/f_{II}$ versus $1/a_{H^+}$. *Slope of straight line obtained is* 4.6×10^2. *The* $K^{*'}_{II-I}$, *as calculated from this value, equals* 1.13×10^6 M^{-1}.

The effect of replacing an amino hydrogen with a methyl group was likewise determined. To this end, VII*a* was prepared, and preparation of VIII was attempted. Attempts to isolate VIII as a pure compound were unsuccessful, the end-product being contaminated with VII*a* and other impurities. Nevertheless, studies done as before, using impure material, indicated that VIII was rapidly and completely hydrolyzed in aqueous solution, irrespective of pH. Corresponding studies with VII*a*, which was isolable as a pure compound, confirmed these observations in that no significant ring formation was observed.

The situation in this case is somewhat different than the general case because free base and protonated forms for the diazepinone no longer exist in equilibrium. The reaction involved can be represented by Scheme IV.

$$\text{VII}b \overset{K_a^{\text{VII}b}}{\rightleftharpoons} \text{VII}a + H^+ \overset{K^{*'}_{\text{VII-VIII}}}{\rightleftharpoons} \text{VIII} + H_2O$$

Scheme IV

The following equation, derived as was Eq. 5, would then relate $f_{\text{VII}a}$ to hydrogen-ion activity:

$$f_{\text{VII}} = \frac{K_a^{\text{VII}b} + [H^+]}{K_a^{\text{VII}b} + [H^+](1 + K_a^{\text{VII}b} K^{*'}_{\text{VII-VIII}})} \qquad \text{(Eq. 11)}$$

Equation 11 has the following limiting forms:

$$\lim f_{\text{VII}}^{H^+ \to \infty} = \frac{1}{1 + K_a^{\text{VII}b} K^{*'}_{\text{VII-VIII}}} \qquad \text{(Eq. 12)}$$

$$\lim f_{\text{VII}}^{H^+ \to 0} = 1 \qquad \text{(Eq. 13)}$$

As seen by examination of Eq. 12, for measurable coexistence of both open

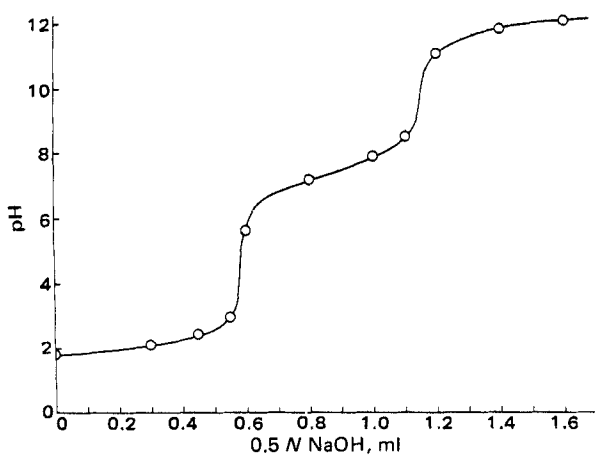


Figure 5—*Plot of initial pH values obtained by extrapolation for partially neutralized solutions of II*c.

Table III—Dissociation Constants (27°)

Compound	pKa_1	K_{a_1}	pKa_2	K_{a_2}
I*b*[a]	3.54 ± 0.03	2.89×10^{-4}	11.4 ± 0.05	3.98×10^{-12}
II*b*[b]	Very low	Very high	7.39 ± 0.06	4.07×10^{-8}
V*b*[a]	3.42 ± 0.05	3.80×10^{-4}	—	—
VII*b*[b]	Very low	Very high	7.74 ± 0.05	1.82×10^{-8}

[a] Determined spectrophotometrically. [b] Determined titrimetrically.

and closed forms to occur, the term $K_a^{\text{VII}b} K^{*'}_{\text{VII-VIII}}$ must be significant compared with 1. The value of $K_a^{\text{VII}b}$ (Table III) is somewhat less than the value of $K_{a2}^{\text{II}b}$. If $K^{*'}_{\text{I-II}}$ were equal to $K^{*'}_{\text{VII-VIII}}$, this decrease in K_a would account for an increase in the value of the limit in very acidic solution from 0.96 (as it is for the II–I system) to 0.98. It is apparent then that the values of $K^{*'}_{\text{II-I}}$ and $K^{*'}_{\text{VII-VIII}}$ need not be different, despite the fact that f_{VII} is independent of pH and near unity. Certainly, no increase in the value of the ring closure equilibrium constant occurs. The primary reason for the observed difference in behavior between the II–I and VII–VIII systems is that no storage of an unreactive form of the pyrazolodiazepinone can occur in the VII–VIII system whereas storage in the form of unprotonated I is allowed in the II–I system.

CONCLUSIONS

At near ambient temperatures, I*a* and its 4-*N*-methyl derivative (V*a*) undergo facile reversible hydrolysis in aqueous solvents of pH 7.0 or less. Under these conditions, equilibrium mixtures consisting of the unhydrolyzed diazepinones and corresponding open forms II and VI are produced. The composition of equilibrium mixtures depends on the pH of the reaction solution and has been shown to be related to the hydrogen-ion concentration according to:

$$f_{\text{open}} = \frac{K_a^{\text{open}} + [H^+]}{K_a^{\text{open}} + [H^+] + K_a^{\text{open}} K^{*'} (K_a^{\text{closed}} + [H^+])} \qquad \text{(Eq. 14)}$$

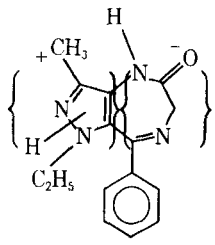
The values of $K^{*'}$, the intrinsic closure constants for II*a* and VI*a*, were calculated to be 1.13×10^6 and 6.1×10^6, respectively. Methylation of the amido nitrogen of II*a* caused a significant increase in the value of the ring closure equilibrium constant. Methylation of the terminal amino group caused no corresponding increase and may not significantly influence equilibrium.

APPENDIX

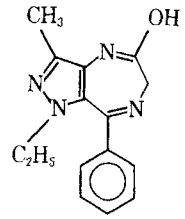
Dissociation Constants and Sites of Protonation—Other properties associated with I and II relate to this study and require determination. Among these are dissociation constants and protonation sites. This information is pertinent to this equilibrium study as well as to a kinetic investigation to be reported later. In regard to the open form, II, the determination of dissociation constant(s) is complicated by its instability. As has been established, II converts to I or equilibrium mixtures containing I in aqueous solution at 27°. To cope with this situation, the initial pH resulting from partial neutralization of the dihydrochloride salt of II*a* was determined by extrapolation (see *Experimental*). The pKa of II*a* (Table III) was then calculated using pH values defined by the smooth curve (Fig. 5) drawn through these extrapolated pH values.

As seen from Fig. 5, II*c* has two well-separated pKa values. The pH's observed during addition of up to 1 mole of sodium hydroxide/mole of the II*a* dihydrochloride salt were the same as those obtained when an equimolar solution of hydrochloric acid was similarly titrated. The UV spectra of II*a*, determined in hydrochloric acid solutions, showed no significant difference in cases where the concentration of hydrochloric acid was 1.0 *N* or less (Table II). The pKa of 1,3-dimethylpyrazole was reported to be 3.11 (12). Since the 1,3-dialkylpyrazole ring present in II*a* contains the benzoyl substituent, its pKa would be expected to be lower than that of 1,3-dimethylpyrazole. The presence of a positive charge would cause a further diminution of the pKa. These considerations indicate that the first dissociation of diprotonated II*a* involves proton loss from pyrazole nitrogens. The pKa associated with this process is very low and has a value less than zero.

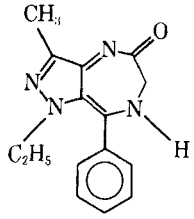
The second pKa, $pKa_2^{\text{II}b}$, is much greater, indicating that a stronger basic site is involved. Since the pyrazole nitrogens have been excluded, this dissociation must involve the primary amino group of the acetamido chain. This conclusion is consistent with pKa values of C-carbamoyl-C-aminomethane compounds whose pKa's are reported to be 8.3–8.8 (13). The pKa of the model carbamoylaminomethane derivatives would be modified by substitution as present in II*a*.



Ic



Id



Ie

Dissociation constants of Ia were determined spectrophotometrically. This procedure is preferred since the spectra of charged and neutral forms are significantly different (Table IV) and the time required for spectrophotometric measurement (with precautions) is short compared with the time required for significant hydrolysis (Table III). The possibility that Ia might behave as a polyfunctional base was considered. Nonaqueous titration of Ia, using acetic acid as solvent and perchloric acid as titrant, indicated that only 1 mole of hydrogen ion was neutralized per mole of compound, so it could not behave as a polyfunctional base in aqueous solution over the pH region of interest. Compound Ia is, however, amphoteric. The second dissociation of Ib involves deprotonation from the diazepinone nitrogen in the 4-position since the 4-*N*-methyl derivative of Ia, Va, does not dissociate accordingly.

Several tautomeric structures can be written to represent other species of Ia with no net charge that might exist in aqueous solution. Significant concentration of forms Ic–Ie, however, cannot exist because the UV properties of Va (for which these tautomers could not exist) and Ia are very similar (Table II).

Evidence for the site of protonation of Ib is also available from comparison of its UV spectrum and that obtained from suitably methylated derivatives. The spectrum of Ib was significantly different from that of Ia and was characterized by absorption at near 300 and 371 nm (Table II). Compound IX (see *Experimental*), which contains methyl groups on both 4- and 7-nitrogens, was prepared and had a UV spectrum with essentially the same shape as Ib with maxima at nearly the same wavelengths (Table II). This result indicates that protonated Ia has the structure shown as Ib. Apparently, Ia behaves as do other benzodiazepinones in regard to proton addition (14).

Derivation of f_{II}—Let:

$$T = [IIa] + [IIb] + [Ia] + [Ib] \qquad \text{(Eq. A1)}$$

Then:

$$f_{II} = \frac{[IIa] + [IIb]}{T} \qquad \text{(Eq. A2)}$$

$$f_{II} = \frac{1 + \dfrac{[IIb]}{[IIa]}}{1 + \dfrac{[IIb]}{[IIa]} + \dfrac{[Ia]}{[IIa]} + \dfrac{[Ib]}{[IIa]}} \qquad \text{(Eq. A3)}$$

$$f_{II} = \frac{1 + \dfrac{[H^+]}{K_{a2}^{IIb}}}{1 + \dfrac{[H^+]}{K_{a2}^{IIb}} + K_{a1}^{Ib}K^{*\prime} + K^{*\prime}[H^+]} \qquad \text{(Eq. A4)}$$

$$f_{II} = \frac{K_{a2}^{IIb} + [H^+]}{K_{a2}^{IIb} + [H^+] + K^{*\prime}K_{a2}^{IIb}K_{a1}^{Ib} + K_{a2}^{IIb}K^{*\prime}[H^+]} \qquad \text{(Eq. A5)}$$

$$f_{II} = \frac{K_{a2}^{IIb} + [H^+]}{K_{a2}^{IIb} + [H^+] + K_{a2}^{IIb}K^{*\prime}(K_{a1}^{Ib} + [H^+])} \qquad \text{(Eq. A6)}$$

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[x] To whom inquiries should be directed.