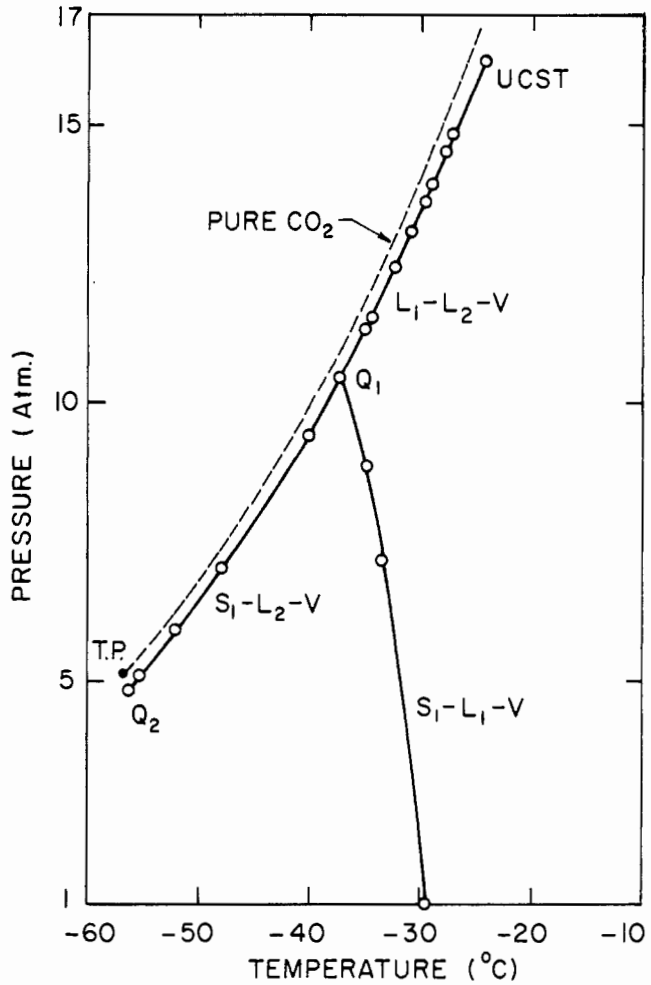

Figure 1. Phase equilibria in system CO_2–*n*-decane (*P-T* projection). Pure CO_2 data denoted by dashed line

curately along this locus. The authors feel that this locus should rise from Q_1 with a slight positive slope.

The raw data were smoothed by plotting them on a large scale graph paper. The standard deviations of the raw data from the smoothed values of pressure, composition, and liquid molar volume are ±0.2 atm, ±0.002 mole fraction, and ±0.2 cc/g-mol, respectively.

Nomenclature

L_1 = CO_2 lean liquid phase
L_2 = CO_2 rich liquid phase
P = pressure, atm
Q_1 = quadruple point 1 (coexistence of the four phases $V-L_1-L_2-S_1$)
Q_2 = quadruple point 2 (coexistence of the four phases $V-L_2-S_1-S_2$)
S_1 = solid *n*-decane
S_2 = solid CO_2
T = temperature, °C
UCST = upper critical solution temperature of L_1 and L_2
V = vapor phase
$\bar{V}$ = molar volume, cc/g-mol
X = composition

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Received for review July 18, 1973. Accepted October 15, 1973. Apparatus constructed under grants from the National Science Foundation. Work supported by National Science Foundation Grant No. GK 36490X.

Stability of First Dissociable Proton of Uric Acid

Birdwell Finlayson[1] **and Arthur Smith**
Division of Urology, Department of Surgery, University of Florida, College of Medicine, Gainesville, Fla. 32610

The stability constant of the first dissociable proton of uric acid was measured at 20°, 25°, 38°, and 45°C by two different techniques—solubility and spectrophotometric titration—with good agreement of results. The spectrophotometric data indicated a p*K*a of 5.468 at 38°C and an enthalpy of ionization of −5.22 kcal/mol. On the basis of observations of sodium acid urate solubility and the internal consistency of the stability measurement data, it was concluded that the sodium urate complex is negligible in biological systems.

The currently accepted (and only reported) value of the p*K*a of uric acid is that of Bergmann and Dikstein (*2*). It was determined at room temperature by plotting the trajectory of the optical absorbance maximum as a function of pH in uric acid in solutions containing various sodium salt buffers. An algorithm for reducing the trajectory data was not given either by Bergmann and Dikstein or by Robinson and Pekrul (*5*), whom they cited. The optical absorbance maximum trajectory as presented by Bergmann and Dikstein had the characteristic shape of a titration curve, and the midpoint of the trajectory or its inflection point was probably used to identify the p*K*a (the two points, for practical purposes, appear to be the same).

Use of the midpoint of the trajectory to identify the p*K*a requires that the molar optical absorbance dispersion curves of uric acid and acid urate have facing shoulders that are mirror images of each other. Absorbance dispersion data on uric acid were not presented. Intrepretation of Bergmann and Dikstein's observation is further complicated by the possibility that a sodium acid urate complex is present in significant concentration. Analysis of sodium acid urate solubility data by Hammarsten (*3*) suggests

[1] To whom correspondence should be addressed.

that a sodium acid urate complex does form and that its thermodynamic stability constant is $\sim 13\ M^{-1}$.

In view of the foregoing considerations, we feel that the p*K*a of uric acid is not known with certainty. We present here measurements of the p*K*a of uric acid, because they are needed in the study of urolithiasis and gout.

Experimental

Water was passed through a charcoal adsorber and mixed resin ion exchanger before use. Uric acid was prepared as described by Porter (*4*). The molar extinction coefficient of this preparation in 0.067*M* glycine buffer (pH, 9.3) at 292 nm is $(1.295 \pm 11) \times 10^4$, as determined by linear regression on 10 independent calibration curves. Sodium acid urate was prepared by the method of Hammarsten (*3*). All other chemicals were reagent grade.

The pH was determined with glass electrodes in thermostatically controlled specimen containers with the temperature within ±0.25°C of the nominal temperature. The electrodes were calibrated with commercially prepared buffers before and after sample measurement. Potentials were measured with either an Orion 801 digital pH meter or a Sargent-Welch Autotitration Model PHR recording potentiometer.

The total dissolved urate was determined spectrophotometrically, as described by Porter (*4*); total dissolved sodium was determined with a Beckman atomic absorption spectrophotometer with an acetylene–air flame (λ = 598 nm).

Solubility studies were done in a constant-temperature water-bath shaker. Approximately 50 mg of solid phase and 15 ml of solution were placed in ground glass stoppered 25-ml Erlenmeyer flasks. The pH was adjusted with HCl or NaOH. To prevent bacterial contamination, it was necessary to heat-sterilize the systems, as described by Allen et al. (*1*). As indicated by approach-to-equilibrium studies, uric acid was allowed to equilibrate for 7 days, and sodium acid urate for 3 days. Equilibrated uric acid supernatant solution was obtained by directly aspirating the supernatant through a 220-nm Millipore filter. Supernatant solution from sodium acid urate equilibrium systems was aspirated through a 25-nm Millipore filter. The aspirates were analyzed for pH, total urate, and sodium when appropriate.

Spectrophotometric titration of uric acid was carried out with 350 ml of a 0.1*M* NaCl, 5-m*M* sodium acetate, and $\sim 5 \times 10^{-5}$ M uric acid solution in a 500-ml closed glass reaction vessel kept within ±0.25°C of its nominal temperature and mixed with a magnetic stirring bar. The pH was monitored with a glass pH electrode and recording potentiometer. During the titration the contents of the reaction vessel were continuously circulated through a thermostatically controlled flow-through cell (0.5-cm light path) in a Gilford Modified Beckman DU spectrophotometer. Optical density was measured at 295 nm. To start the titration, the pH was adjusted to 11 with several drops of 1*M* NaOH; then, 2*M* HCl was added with an automatic titrator. To avoid problems of synchronization of the pH and the optical density trace on the strip chart recorders, HCl was added in pulses. Curves of optical density as a function of pH were constructed from the strip chart records.

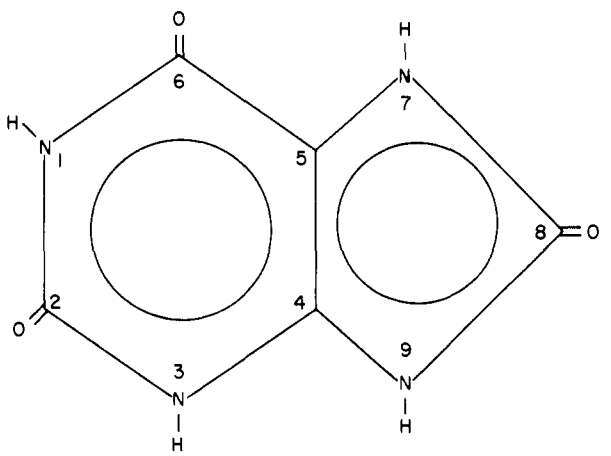

Figure 1. Structural diagram of uric acid

The dispersion of optical absorbance of uric acid (pH, 2) and acid urate (pH, 8.3) was observed in a Cary 14 spectrophotometer with thermostatically controlled sample compartments.

Data Reduction

Numerical analysis of uric acid solubility was carried out assuming the following equilibria:

$$HU_s \rightleftharpoons HU$$

and

$$HU \overset{K_1}{\rightleftharpoons} H^+ + U^-$$

in which HU_s is solid-phase uric acid, HU is dissolved uric acid, and U^- is acid urate. Figure 1 shows the structure of uric acid. Bergmann and Dikstein suggested that the first dissociable proton comes from position 9 (*2*). The mass action equation yields

$$[U_t] = \frac{[HU]}{K_1(H^+)f} + [HU] \qquad (1)$$

in which expressions enclosed in square brackets are molar concentrations, (H^+) is proton activity, U_t is the sum of U^- and HU, and f is calculated as

$$f = \exp\left[-2.303\, A\left(\left(\sqrt{I}/(1+\sqrt{I})\right) - 0.286\, I\right)\right] \qquad (2)$$

in which I is ionic strength, and A is tabulated according to temperature (*6*). The number −0.286 was obtained by transforming 38° molal activity coefficients (*6*) to molar activity coefficients for $CaCl_2$ and NaCl and then making a least-squares fit of the molar activity coefficients to Equation 1.

Equations 1 and 2 can be solved by iterative substitution of approximate solutions and were used in combination as a function of pH and added ions to calculate the concentration of total dissolved urate, $[U_t]$. This function was used in a nonlinear least-squares program to estimate K_1, the thermodynamic stability constant for the first dissociating proton of uric acid. The minimal value of total urate concentration was taken from a plot of total urate concentration vs. pH (Figure 2).

Sodium acid urate solubility as a function of added sodium chloride (ANa) was calculated with

$$[U_t] = \frac{-[ANa] + ([ANa]^2 + (4K_3(1 + K_1 f(H^+))/f^2))^{1/2}}{2(1 + K_1 f(H^+))} \qquad (3)$$

which can be solved by iteration and was used as a function of pH and added ions in a nonlinear least-squares program to estimate the solubility product constant (K_3) for sodium acid urate. Equation 3 assumes that no sodium acid urate complex is formed.

To analyze the spectrophotometric titration of uric acid, we define the variable θ_j:

$$\theta_j = (\Delta OD_t - \Delta OD_j)/\Delta OD_t \qquad (4)$$

in which ΔOD_t is the maximal optical density change resulting from complete conversion of acid urate to uric acid [in practice, this is the difference in optical density between a pH of 8.2 and the plateau extending beyond a pH of 3.5 (Figure 3)], and ΔOD_j is the difference in optical density between a pH of 8.2 and the pH after the jth increment of acid. The optical density differences were corrected ($\leq$1.5%) for the volume change owing to titrant. It follows from the definition of θ_j that

$$\log K_1 = \text{pH} + \log\left(\frac{1-\theta_j}{\theta_j}\right) - \log f \qquad (5)$$

in which f is the activity coefficient for a univalent ion, and K_1 is the thermodynamic stability constant used in Equation 1.

Results and Discussion

Dispersion of the optical absorbance of uric acid and acid urate is shown in Figure 4, in which the absorption curves are not symmetrical about their maxima, and the optical densities of the maxima are not equal. Asymmetry of the uric acid and acid urate absorbance dispersion curves about their maxima and inequality of the maxima indicate that the technique used by Bergmann and Dikstein (*2*) to determine the first p*K*a of uric acid is not strictly valid, and their results need not be rationalized with our results. Our measurements indicate that λ_{max} (uric acid) = 283 nm with ϵ (uric acid) = 12.54 × 10^3 and λ_{max} (acid urate) = 292 nm with ϵ (acid urate) = 12.95 × 10^3.

The solubility of sodium acid urate in NaCl solutions is shown in Figure 5. On the basis of Equation 3, our least-squares fit determination of the solubility product constant for sodium acid urate (K_3) is (3.74 ± 0.10) × 10^{-5} M^2. The major solid line in Figure 5 was calculated with the logarithm of Equation 3, by use of our estimates of K_3 and K_1. The failure of the solubility of sodium acid urate to tend toward a constant value at high sodium concentration suggests that significant concentrations of a sodium acid urate complex do not exist in our systems. Our results agree with the observations of Allen et al. (*1*) but not with those of Hammarsten (*3*). We suspect that the difference may be due to inadequate filtration in Hammarsten's system.

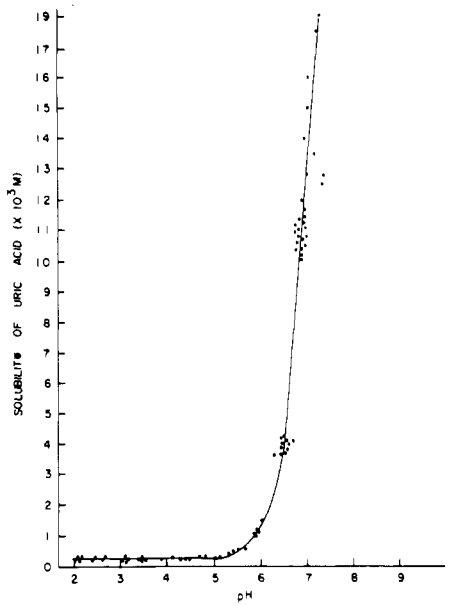

Figure 2. Solubility of uric acid as function of pH at 38°C. Solid line is least-squares fit of data of Equation 1

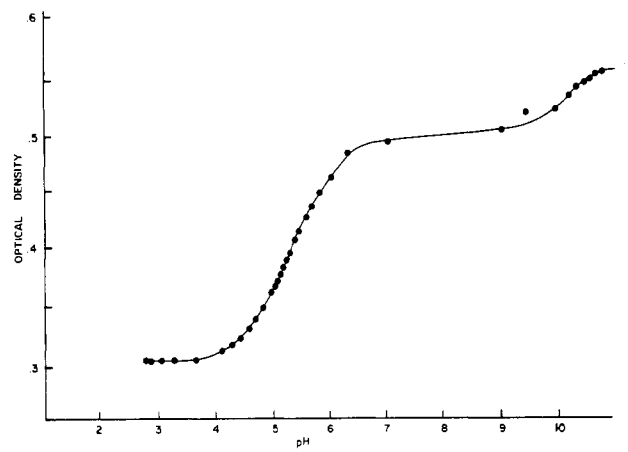

Figure 3. Representative spectrophotometric titration of 5 × 10^{-5} M uric acid with HCl at 38°C in 0.1*M* NaCl and 0.005*M* $NaC_2H_3O_2$ solution. λ = 295 nm

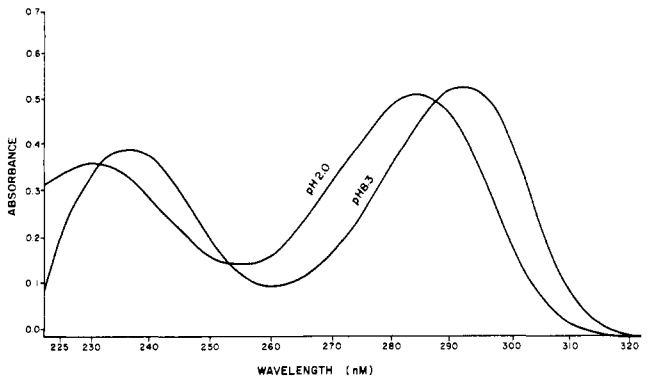

Figure 4. Optical absorbance dispersion of uric acid and sodium acid urate. Concentration of uric acid and acid urate, 0.042 m*M* at room temperature. Scan made at 38 ± 0.1°C with Cary 14 spectrophotometer with 1-cm light path, minimal slit height, and servoadjusted slit width. Read against 38°C water blank by use of matched quartz cuvettes. Uric acid adjusted to pH 2.0 with 0.025 m*M* perchloric acid. Acid urate scanned in 0.05*M* tris (hydroxymethyl aminomethane) buffer with pH adjusted to 8.3 with 1*N* HCL

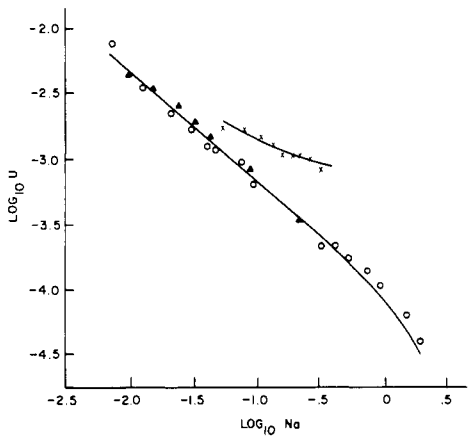

Figure 5. Logarithm of solubility of sodium acid urate (U) as function of logarithm of added sodium concentration at 38°C. Nominal pH is 6.34 ± 0.10 for our observations (circles). Also shown are observations by Hammarsten (*3*) (x) and Allen et al. (*1*) (solid triangles). Line through Hammarsten's observations drawn arbitrarily. Line associated with our measurements is least-squares fit of Equation 3 to our data

The solubility of uric acid at 38°C as a function of pH is shown in Figure 2. On the basis of 72 observations and assuming negligible sodium acid urate complex formation, the stability constant for the first proton dissociable from uric acid (K_1) was estimated with Equation 1 at

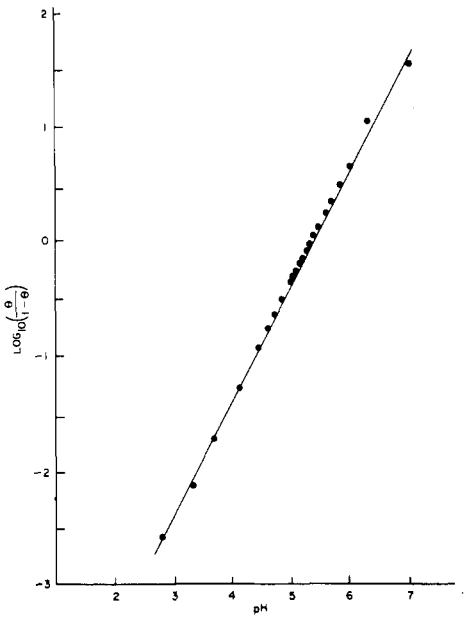

Figure 6. Linear transformation of data in Figure 3 according to Equation 5

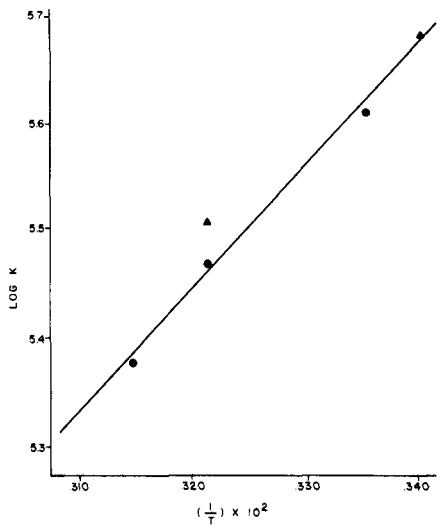

Figure 7. Arrhenius plot of our measured values of K_1 obtained by solubility measurement (solid triangles) and by spectrophotometric titration (dots). Measurement of Bergmann and Dikstein (*2*) (corrected for ionic strength) does not lie in range of this graph

Table I. Summary of Estimates of Log K_1

	Log K_1	
Temp, °C	Solubility	Spectrophotometric titration
20	5.657	...
Room temp[a]	...	5.820
25	...	5.607
38	5.509	5.468
45	...	5.378

[a] From ref. **2** and corrected for ionic strength.

(3.23 ± 0.23) × 10^5 M^{-1} and the minimal solubility of uric acid, at 0.257 ± 0.006 m*M*. For 45 similar observations made at 20°C, the minimal solubility of uric acid was 0.146 ± 0.002 m*M*, and K_1 was (4.54 ± 0.61) × 10^5 M^{-1}.

A representative example of an optical titration curve is shown in Figure 3. A plot of data in Figure 3 transformed according to Equation 5 is shown in Figure 6, where the linearity persists from a pH of 8.2 to a pH of less than 3. By calculating with Equations 5 and 2 and 90 observation points from six titrations at 38°C, we estimated log K_1 at 5.47 ± 0.02. By using 44 observations from three titrations at 25° and 45°C, we estimated log K_1 at 5.61 ± 0.02 and 5.38 ± 0.02, respectively. Estimates of log K_1 are summarized in Table I.

Figure 7 shows the temperature dependence of K_1. From the plot, the spectrophotometric and solubility techniques yield consistent results. The slope of the line in Figure 7 indicates that the enthalpy of ionization is −5.22 kcal/mol. The linearity of the plot of titration data in Figure 6, the fidelity of calculated to observed sodium acid urate solubility over 4 orders of magnitude of sodium concentration (Figure 5), and the close agreement of the stability constant estimates resulting from two very disparate methods (Table I and Figure 7) appear to justify the assumption used in our calculations that a sodium acid urate complex does not form. Furthermore, it is reasonable to assume that the complex does not form in systems of biological interest.

In serum the predominant species is acid urate. The calculated serum acid urate concentration difference between the Bergmann and Dikstein value (*2*) and our value is only 1%. However, in human urine, uric acid is the species of predominant interest; at a pH of 6 with an ionic strength of 0.15, the p*K*a of Bergmann and Dikstein predicts 87% more undissociated uric acid than our value predicts.

Nomenclature

A = temperature-dependent term used in calculating activity coefficients; see Robinson and Stokes (*6*) for table of values
ANa = NaCl added to a solubility equilibrium system
f = single ion monovalent activity coefficient
HU = uric acid in solution
HU_s = solid phase uric acid
K_1 = stability constant for the first dissociable proton on uric acid
K_3 = solubility constants for sodium acid urate
U or U^- = dissolved acid urate
U_t = sum of all dissolved uric acid and acid urate
ΔOD = change in optical density
ϵ = molar extinction coefficient, 38°C, 1-cm path length
λ_{max} = wavelength of maximum optical density
θ_j = relative change in optical density following the jth increment of acid
$[x]$ = molar concentration of x
(x) = molar activity of x

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Received for review July 18, 1973. Accepted October 26, 1973. Research supported by the U.S. National Institutes of Health Grant No. AM-13023-04.