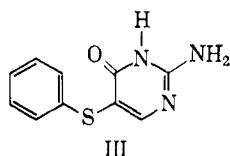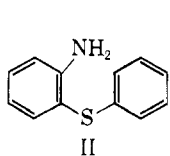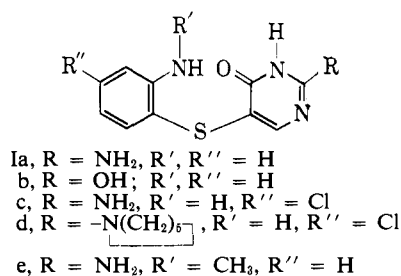# 5-Arylthiopyrimidines. IV. Spectrophotometric Determination of Successive Acid Dissociation Constants Differing by Less Than Two pK Units

Barbara Roth and J. F. Bunnett

Contribution from The Wellcome Research Laboratories, Burroughs Wellcome and Company, Tuckahoe, New York, and The Metcalf Chemical Laboratory, Brown University, Providence, Rhode Island. Received August 24, 1964

Evidence is presented that 2-amino-5-(o-aminoaryl-thio)pyrimidin-4-ones such as Ia undergo protonation at either and then both of two basic sites within the pH range 0–6. The two stages of protonation are associated with very similar changes in spectra. From photometric data, by means of an electronic computer and with use of equations developed by Thamer, pK_a values for two stages of protonation which differ by only 1.4–1.7 pK units have been evaluated for four compounds. Apart from its organic chemical interest, this perhaps constitutes the first successful application of Thamer's methods to practical cases with inconvenient spectral relationships. pK_a values for the acid dissociation of these and related compounds in alkaline media have also been determined.

In a study of the kinetics of the acid-catalyzed cyclization of 5-(o-aminoarylthio)pyrimidin-4-ones[1] (I) to 10H-pyrimido[5,4-b][1,4]benzothiazines[2] it became necessary to know the pK_a values for the pyrimidines of structure I. Since the ultraviolet spectra of the different ionic forms of these ampholytes are quite different,[3] a satisfactory means was at hand for the investigation of their ionization equilibria.

Ia, R = NH₂, R', R'' = H
b, R = OH; R', R'' = H
c, R = NH₂, R' = H, R'' = Cl
d, R = -N(CH₂)₅-, R' = H, R'' = Cl
e, R = NH₂, R' = CH₃, R'' = H

II   III

In the case of Ib, determination of the pK_a values for the gain and for the loss of one proton was a routine matter. Good isosbestic points were defined both by the family of spectral curves at pH 0–5 (proton gain) and by the family at pH 6–11 (proton loss). Plots of optical density at a given wave length, or of the difference between optical densities at two wave lengths where large differences occur, *vs.* pH were sigmoid and matched

the curves calculated for pK_a equal to the pH at the inflection point[4] (Figure 1). The pK_a representing protonation of the base was found to be 2.99. This undoubtedly represents protonation of the anilino nitrogen. 5-Phenylthiopyrimidine-2,4-dione,[2] which is like Ib but lacks its amino group, undergoes no change in spectrum between pH 0 and 6. The pK_a of Ib as an acid was 8.30; this shows the substance to be slightly more acidic than uracil (pK_a 9.38).[5]

Determination of the acid dissociation constants for Ia and Ic–Ie in alkaline solution was also uncomplicated. Spectral changes were not large, but good isosbestic points were defined. The greatest spectral change was observed with Id, representative curves for which are presented in Figure 2.

Investigation of the protonation equilibria of Ia and Ic–Ie in acid solutions presented more of a problem. The compounds were all found to undergo shifts in ultraviolet absorption spectra between pH 0 and 6 similar to that of Ib, but in none of the four spectral series did the families of curves in this pH range define isosbestic points. This is illustrated in Figure 3 for Ia, which is representative of the four compounds. This lack of isosbestic points indicates the presence of pH-dependent equilibria between more than two species. The possibilities that Ia or its protonated form at pH 0 do not follow Beer's law were disposed of by direct experiments. The possibility that a buffer constituent (acetate or phosphate ion) was associating with the heterocyclic compound to form a third species, perhaps by hydrogen bonding with the 4-oxo and/or amino groups, was eliminated by demonstration that within experimental error a change in buffer did not change the spectra at a given pH, nor indeed did complete elimination of a buffer, providing the pH was the same. One may also deduce that interactions with the 4-oxo and o-amino groups are not responsible for the lack of isosbestic points from the fact that Ib does not exhibit this phenomenon.

Evidence of Diprotonation. Plots of extinction coefficient, or of the difference in extinction coefficients at two wave lengths where they were quite different,[4] against pH were approximately sigmoid and at first glance similar to those for Ib. However, these curves showed small irregularities in the middle of the steep portions. Moreover, it was not possible to reconstruct the experimental curves adequately on the assumption of a single stage of protonation with pK_a equal to the pH at the middle of the steepest portion. This is demonstrated in Figures 4 and 5 for the cases of Ia and Ic.

(1) These pyrimidines are named according to the format in *Advan. Heterocyclic Chem.*, 1, 315 (1963).

(2) B. Roth and J. F. Bunnett, *J. Am. Chem. Soc.*, 87, 340 (1965).

(3) B. Roth and L. A. Schloemer, *J. Org. Chem.*, 28, 2659 (1963) (part III of this series).

(4) C. T. Davis and T. A. Geissman, *J. Am. Chem. Soc.*, 76, 3507 (1954).

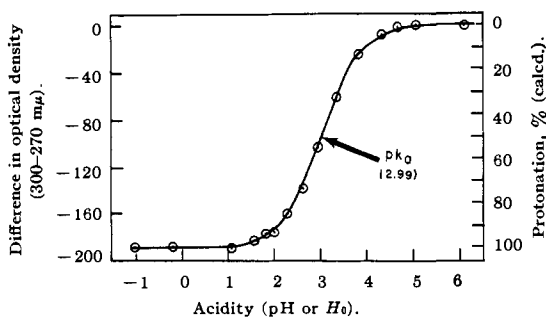(5) A. Albert and J. N. Phillips, *J. Chem. Soc.*, 1294 (1956).

Figure 1. 5-(o-Aminophenylthio)pyrimidin-2,4-dione (Ib): $(O.D._{.300} - O.D._{.270})$ vs. acidity (pH or H_0); open circles, experimental points; solid curve, calculated for $pK_a = 2.99$.
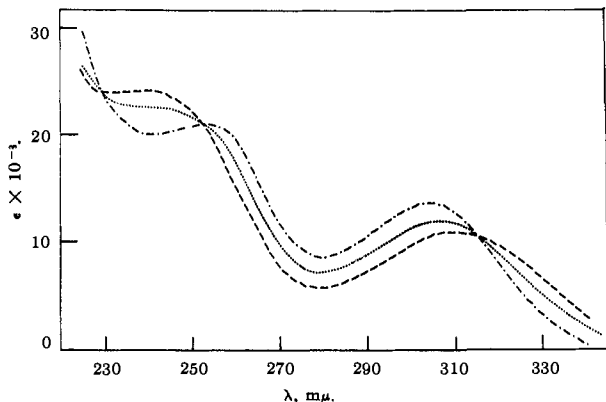

Figure 2. 5-(2-Amino-4-chlorophenylthio)-2-piperidinopyrimidin-4-one (Id): – – – –, ultraviolet absorption spectra of neutral species (pH 5.7); —·—·—, anion (pH 13); ······, intermediate (pH 8.48).
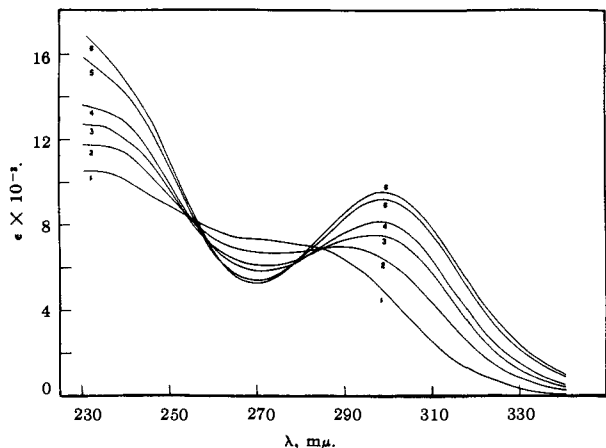

Figure 3. 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia): ultraviolet absorption spectra between pH 0–6; 1, pH 0; 2, pH 2.01; 3, pH 2.59; 4, pH 3.00; 5, pH 4.09; 6, pH 6.08.

The structures of compounds Ia and Ic–Ie possess two basic moieties, either or both of which could conceivably receive a proton. These moieties are separated by a thioether bridge which should act as an insulator. For this and the above reasons, it seemed probable that two stages of protonation occurred in the pH range 0–6. In the case of Ic, further evidence in support of this view is that the analysis of the hydrochloride which crystallized from 3 M hydrochloric acid gave (silver nitrate titration) 1.7 moles of ionic chlorine.
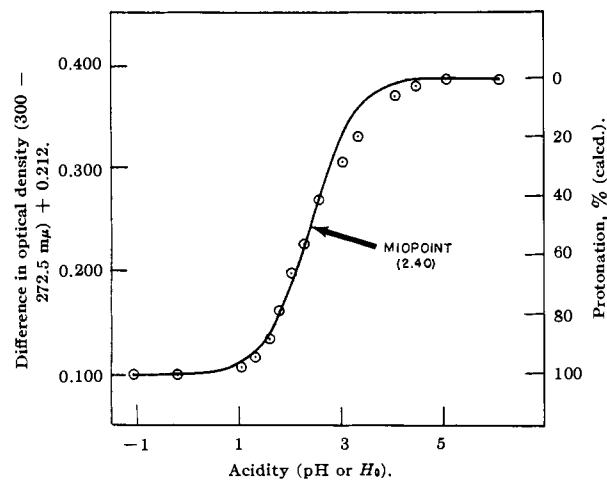

Figure 4. 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia): $(O.D._{.300} - O.D._{.272.5})$ vs. acidity (pH or H_0); open circles, experimental points; solid curve calculated for a single stage of protonation with $pK_a = 2.40$.

Figure 5. 2-Amino-5-(2-amino-4-chlorophenylthio)pyrimidin-4-one (Ic): $(O.D._{.302.5} - O.D._{.272.5})$ vs. acidity (pH or H_0); open circles, experimental points; solid curve calculated for a single stage of protonation with $pK_a = 2.17$.
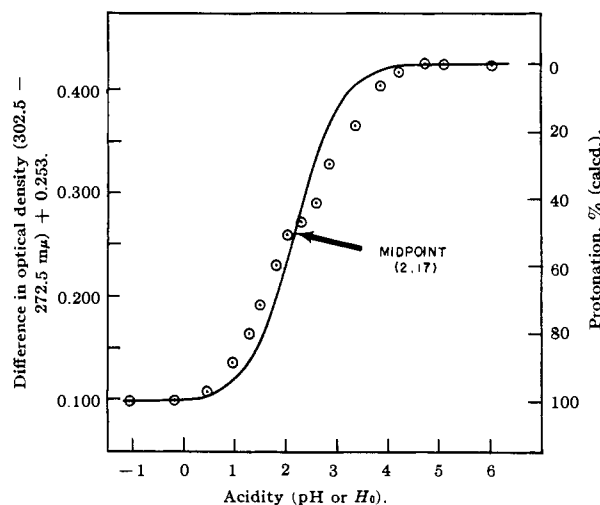
Model Compounds. In an effort to ascertain what pK_a values might be expected for the protonation of each moiety of Ia, two model compounds were prepared. The first of these, o-phenylthioaniline (II), was found to have a pK_a of 2.5. The second compound, 2-amino-5-phenylthiopyrimidin-4-one (III), has a pK_a of 2.9, representing protonation of the base. Spectra of each neutral and cationic species are shown in Figure 6. The similarity in spectra of the two systems is to be noted. Both compounds show maxima in the same regions. The neutral pyrimidine absorbs more strongly in the 300-mμ region than does the diaryl sulfide, but the latter has a somewhat higher absorption coefficient in the 240-mμ region. The protonated pyrimidine shows generally greater absorption than does protonated o-phenylthioaniline. The spectrum of Ia differs from both of these in the region below 245 mμ, where the maximum undergoes a hypsochromic shift which is almost obscured by increased end absorption below 230 mμ.

Theoretical Considerations. The similarity in pK_a values of II and III suggests that two tautomeric mono-
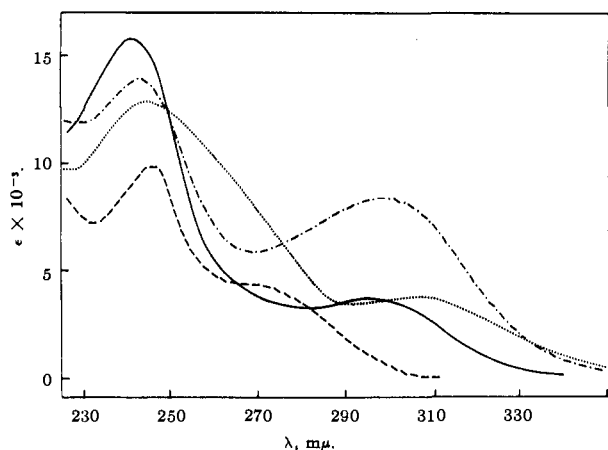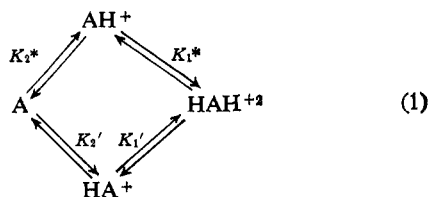
Figure 6. Ultraviolet absorption spectra of o-phenylthioaniline (II): - - - -, cation; ————, neutral species; and 2-amino-5-phenylthiopyrimidin-4-one (III); ·······, cation; —·—·—, neutral species.

protonated forms participate importantly in the protonation of Ia. The situation is then of the type (the hydrogen ions involved are omitted)

$$
\begin{array}{ccc}
 & AH^+ & \\
K_2^* \nearrow & & \searrow K_1^* \\
A & & HAH^{+2} \\
K_2' \searrow & & \nearrow K_1' \\
 & HA^+ &
\end{array}
\qquad (1)
$$

In such a case, the experimental dissociation constants, K_1 and K_2, are composite. It is easily shown that

$$K_1 = K_1^* + K_1' \qquad (2)$$

$$\frac{1}{K_2} = \frac{1}{K_2^*} + \frac{1}{K_2'} \qquad (3)$$

Only if K_1^* is much greater or much less than K_1' (differing by, say, 100-fold or more) does the experimental pK_1 or pK_2 approximate that for protonation of a single site.

It is also easily shown that $[AH^+]/[HA^+] = K_2'/K_2^*$. Stated in words, the ratio of concentrations of the two monoprotonated forms is constant, regardless of the pH.[6] The apparent extinction coefficient of monoprotonated substrate is therefore the sum of constant fractions of the extinction coefficients of AH^+ and HA^+. Consequently, "monoprotonated A" behaves spectrally as though it were a single species. The failure of the curves in Figure 3 to define isosbestic points thus cannot be attributed to the participation of two different monoprotonated species in the equilibrium.

Both the similarity in pK_a of II and III and the shape of the curves in Figures 4 and 5 indicate that the two stages of protonation of Ia and Ic–Ie overlap each other. Direct determination of the apparent extinction coefficients of the monoprotonated forms is therefore precluded. To determine pK_1 and pK_2, a more sophisticated approach must be employed.

Thamer's Methods. The general problem of determining dissociation constants of dibasic acids from

spectrophotometric data has been treated by Thamer.[7] The basic relationship, which is easily derived, is

$$
D = \frac{Lc\left[E_1 + \dfrac{K_1 E_2}{[H^+]} + \dfrac{K_1 K_2 E_3}{[H^+]^2} \right]}{1 + \dfrac{K_1}{[H^+]} + \dfrac{K_1 K_2}{[H^+]^2}}
\qquad (4)
$$

where K_1 and K_2 are the measured dissociation constants, D is the measured optical density, L is the length of the absorption cell, c is the total concentration of the base in all its forms, and E_1, E_2, and E_3 are the molar extinction coefficients of diprotonated, monoprotonated, and neutral A, respectively. This is an equation of five unknowns (K_1, K_2, E_1, E_2, and E_3) and two experimentally measurable quantities, $[H^+]$ and D. One should be able to solve for all five unknowns if five simultaneous equations are assembled, each representing a different hydrogen ion concentration and optical density value (at a single wave length). Thamer recommended that for best accuracy the hydrogen ion concentrations of the five measured solutions (designated α, β, γ, δ, and ϵ) should approximate those at which concentrations of the di-, mono-, and nonprotonated forms are maximum (α, γ, and ϵ) and those at which the two stages of protonation are half-complete (β and δ).

For solution of the sets of five simultaneous equations, Thamer outlined methods suitable for desk computation. These are tedious and fraught with opportunities for arithmetic error. He applied them to a problem in which the extinction coefficient of the monoprotonated form at the chosen wave length was greater than that of either the di- or nonprotonated form. To our knowledge, never has a problem of this sort been solved in which the monoprotonated form had an intermediate extinction coefficient and in which the two stages of protonation overlapped.

Computer Calculation. We rewrote Thamer's eq. 3 in the form

$$a_i v + b_i w + c_i x + d_i y + e_i z = f_i \qquad (5)$$

where

$$
\begin{aligned}
a_i &= [H^+]_i D_i & v &= K_1 \\
b_i &= D_i & w &= K_1 K_2 \\
c_i &= -1 & x &= D_3 K_1 K_2 \\
d_i &= -[H^+]_i & y &= D_2 K_1 \\
e_i &= -[H^+]_i^2 & z &= D_1 \\
f_i &= -[H^+]_i^2 D_i
\end{aligned}
$$

Values for a_i–f_i were determined experimentally and arranged in vertical rows for processing in the computer to obtain the solutions v–z.

For each of the four compounds (Ia, Ic–Ie), approximately a dozen sets of values were selected for solution by the above method. In the case of Ia, plots of optical density *vs.* acidity function were prepared at four wave lengths where the greatest differences in optical densities occurred as a function of ionization. Various α–ϵ pH selections were made from each plot, following Thamer's suggestions in so far as possible. These represented actual experimental values, not points from smoothed curves. Plots were also constructed using the differ-

(6) This conclusion is valid so long as the activity coefficient ratio, f_{AH^+}/f_{HA^+}, is pH independent, a condition doubtless satisfied in the present experiments.

(7) B. J. Thamer, *J. Phys. Chem.*, **59**, 450 (1955); see also B. J. Thamer and A. F. Voigt, *ibid.*, **56**, 225 (1952); D. H. Rosenblatt, *ibid.*, **58**, 40 (1954); H. Irving, H. S. Rossotti, and G. Harris, *Analyst*, **80**, 83 (1955).

Table I. Spectrophotometric Data and Basic pK_a Values (Thamer Method) for 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia)

Calculation no.	Conditions	pH_t^a (O D., ΔO.D., or area)_t					Roots				
		α	β	γ	δ	ε	pK_1	pK_2	D_1	D_2	D_3
1	Difference, 300–272.5 mμ (ΔO.D. + 0.212)^b	1.05 (0.107)	2.01 (0.195)	2.59 (0.271)	3.33 (0.331)	5.09 (0.385)	2.1	3.7	0.0874^c	0.319^c	0.387^c
2	Single wave length, 300 mμ (O.D.)	1.05 (0.206)	2.01 (0.269)	2.59 (0.320)	3.33 (0.365)	5.09 (0.403)	2.0	3.4	0.191	0.351	0.404
3	Single wave length, 272.5 mμ (O.D.)	−0.20 (0.313)	2.01 (0.286)	2.59 (0.261)	3.33 (0.246)	5.09 (0.230)	2.3	3.8	0.313	0.235	0.231
4	Single wave length, 235 mμ (O.D.)	1.05 (0.459)	2.01 (0.501)	2.59 (0.541)	3.33 (0.596)	5.09 (0.679)	2.0	3.6	0.450	0.552	0.682
5	Single wave length, 225 mμ (O.D.)	1.05 (0.455)	2.01 (0.505)	2.59 (0.559)	3.33 (0.628)	5.09 (0.752)	2.3	3.7	0.445	0.589	0.757
6	Single wave length, 300 mμ (O.D.)	1.34 (0.216)	2.01 (0.269)	2.59 (0.320)	3.33 (0.365)	5.09 (0.403)	2.0	3.4	0.187	0.338	0.406
7	Single wave length, 300 mμ (O.D.)	1.05 (0.206)	1.77 (0.246)	2.30 (0.295)	3.00 (0.346)	5.09 (0.403)	2.1	3.6	0.192	0.347	0.406
8	Single wave length, 300 mμ (O.D.)	1.05 (0.206)	2.01 (0.269)	3.00 (0.346)	3.66 (0.376)	6.08 (0.406)	2.0	3.7	0.191	0.351	0.406
9	Single wave length, 300 mμ (O.D.)	1.34 (0.216)	2 01 (0.269)	3.00 (0.346)	3.66 (0.376)	6.08 (0.406)	2.0	3.6	0.188	0.349	0.406
10	Area, 220–350 mμ (area)	1.05 (0.987)	2.01 (1.07)	2.59 (1.15)	3.33 (1.26)	6.08 (1.41)	2.1	3.5	0.967	1.18	1.41
11	Area, 220–350 mμ (area)	1.05 (0.987)	2.01 (1.07)	3.00 (1.21)	4.09 (1.34)	6.08 (1.41)	2.2	3.8	0.969	1.22	1.42
12	Area, 220–350 mμ (area)	−0.20 (0.963)	2.01 (1.07)	2.59 (1.15)	3.33 (1.26)	6.08 (1.41)	2.1	3.5	0.962	1.17	1.41

^{a} The top numbers in the α–ε columns represent experimental pH values; those beneath in parentheses represent optical densities or areas, as indicated in the column of conditions. ^{b} The number 0.212 is added for convenience to convert ΔO.D. to a positive number; its value is immaterial, since it is cancelled by subtraction in solving eq. 5. ^{c} The constant 0.212 must be subtracted to get $D_{300} - D_{272.5}$.

ence between optical densities at two wave lengths, after Davis and Geissman,[4] and selections were made in the same manner from these plots. Such plots tended to be smoother than those at single wave lengths, since small errors were thus eliminated. Since the plots in the lower wave length regions appeared to have a slightly different shape than those for higher wave lengths,
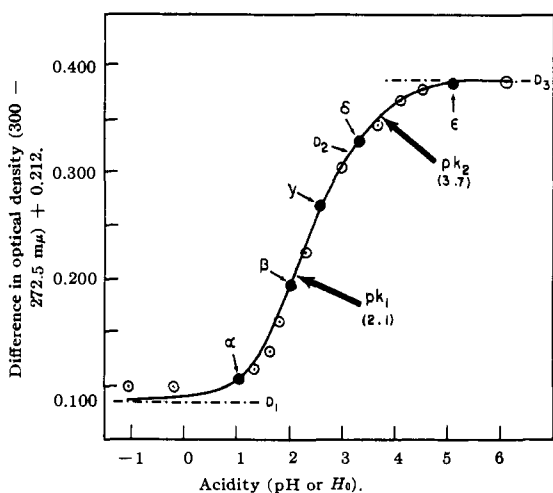
(Since every term of eq. 5 contains an optical density to the first power, using the difference or sum (*i.e.*, area) of optical density values amounts to subtracting or adding corresponding equations of type 5 before feeding values to the computer.)
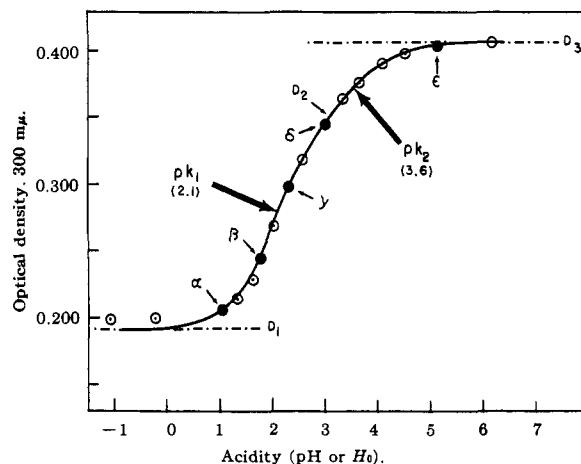

Figure 7. 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia): (O.D.$_{300}$ − O.D.$_{272.5}$) vs. acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots of Table I, calculation 1.

the areas under the spectral curves between 220 and 350 mμ were also determined, for purposes of obtaining an average picture of the changes that occur with pH. Plots of the areas *vs.* acidity function were also roughly sigmoid, and again various α–ε selections were made.

Figure 8. 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia): optical density, 300 mμ, *vs.* acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots of Table I, calculation 7.

The data employed in calculations with Ia and the mathematical solutions or roots obtained are displayed in Table I. Calculation 1 is based on the data of Figure 4, in which the difference in optical densities at two wave lengths is plotted *vs.* acidity function. These data are replotted in Figure 7, along with the theoretical curve obtained by substitution of these roots into eq. 5 at a series of pH values. The reconstructed curve fits
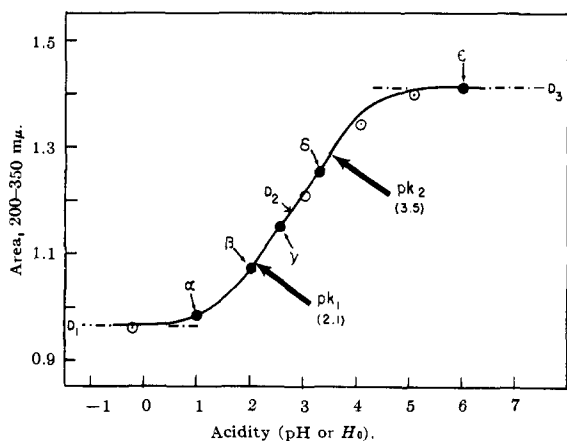
Figure 9. 2-Amino-5-(o-aminophenylthio)pyrimidin-4-one (Ia): area under the optical density curve, 220–350 mμ, vs. acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots of Table I, calculation 10.
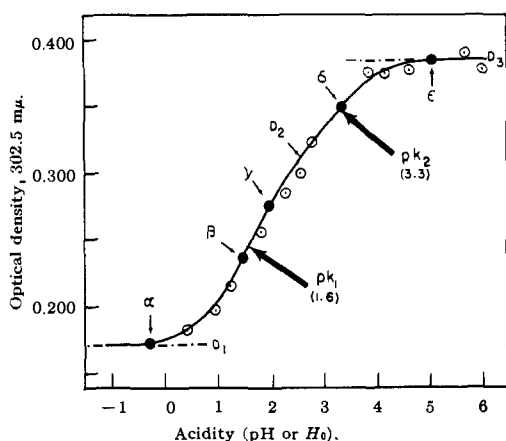

Figure 10. 2-Amino-5-(2-amino-4-chlorophenylthio)pyrimidin-4-one (Ic): optical density, 302.5 mμ, vs. acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots $D_1 - D_3$, pK_1 and pK_2 shown on this graph.

the experimental points quite closely. This contrasts with the poor fit when this process was treated as a monoprotonation (Figure 4).

Calculations 2 and 6–9 of Table I concern various selections of data (representing various sets of pH values) at a single wave length, 300 mμ. The experimental points employed are plotted in Figure 8, together with a curve constructed from the set of roots from calculation 7. It will be noted that the γ-value chosen for this calculation is not close to D_2 (which does not lie very close to the center of the curve). However, in calculations 8 and 9, where γ is very close to D_2, the roots which are pK_a values differ from the corresponding roots in calculation 7 by only 0.1 unit. Thus the selection of the γ-value does not appear to be critical.

Calculations 2, 4, and 5 concern data at three different wave lengths but the same selection of pH values throughout. The pK_a values obtained are virtually the same as from calculations 6–9. Calculation 3 concerns a fourth wave length and a slightly different selection of pH values.
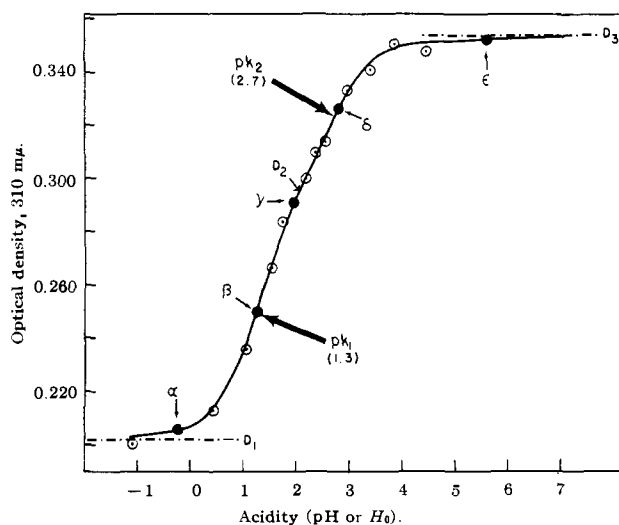

Figure 11. 5-(2-Amino-4-chlorophenylthio)-2-piperidinopyrimidin-4-one (Id): optical density, 310 mμ, vs. acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots $D_1 - D_3$, pK_1 and pK_2 shown on this graph.

Figure 9 depicts the variation in total area under the curves between 220 and 350 mμ as a function of pH. Calculations 10–12 employ selections from these data. The solid line in Figure 9 is constructed from the roots of calculation 10; again it matches the experimental points quite closely. No advantage in accuracy appears to have accrued from the use of areas, however. The areas do not differ very markedly from each other, and the possibility of error in planimeter operation is introduced. In the cases of the other three compounds, the experimental points obtained by use of a planimeter or by averaging the optical densities (at intervals of 2.5 mμ between 220 and 350 mμ) as a function of pH were too erratic to be useful for such calculations.

As seen from Table I, the range of values obtained with Ia for pK_1 is 2.0–2.3, and for pK_2, 3.4–3.8. Variations in these values appear to be dependent chiefly upon the accuracy of the experimental data, and on proper selection of α–ε values. (If the data employed are improperly selected, small differences between large numbers may be subject to a large percentage distortion owing to normal experimental error.)

Certain calculations which were rejected are not displayed in Table I. Some calculations were rejected because they gave nonsense answers (negative equilibrium constants or optical densities) and others because it was not possible to reconstruct from the roots a curve which matched the experimental points. The maximum variation in any pK_a value for any compound among acceptable sets of roots was 0.4 unit.

Similar calculations of pK_1 and pK_2 for compounds Ic, Id, and Ie were performed. For all compounds, we found it desirable to select α-values well out on the flat tail of the curve where diprotonation is virtually complete. When pK_1 was below 2, the α-solution often had a negative H_0 value. In such cases, h_0[8] was used instead of [H⁺] in the calculations. Though we have no assurance that w′ for protonation of these substrates[9]

(8) Values of M. A. Paul and F. A. Long [*Chem. Rev.*, **57**, 1 (1957)] were used for acid concentrations above 0.1 *M*.

is near zero, use of h_0 is acceptable because the calculation is not sensitive to the [H+] employed when the α-point lies on the flat part of the curve.

Results for all compounds are presented in summary in Table II. Figures 10, 11, and 12 depict typical data for compounds Ic–Ie, and show the theoretical curves constructed with use of the roots obtained in the calculations.

Table II. pK_a Values of 5-Arylthiopyrimidines in the pH Range 0–12

	Basic pK_a		
Compd.	pK_1	pK_2	Acidic pK_a
Ia	2.1[b]	3.6[b]	8.7[c]
Ib	2.99[a]	...	8.30[a]
Ic	1.5[b]	3.2[b]	8.6[c]
Id	1.4[b]	2.8[b]	8.5[c]
Ie	2.1[b]	3.6[b]	8.7[c]
5-Phenylthiopyrimi-dine-2,4-dione	...	...	8.13[a]
2-Amino-5- phenylthio-pyrimidin-4-one (III)	2.88[a]	...	8.52[a]

[a] ±0.05 pH unit. [b] Ca. ± 0.2 pH unit. [c] Ca. ±0.1 pH unit.

Curiously, we are unable to specify the pK_a value relating to protonation at pH 0–6 of any of the specific basic moieties in any of the four compounds Ia, Ic–Ie. As mentioned, the K_1 and K_2 values are composite (see eq. 2 and 3). If we knew the ratio [AH+]/[HA+], we could easily reckon the specific values K_1', K_1^*, etc. However, our experiments do not provide the necessary information. We grant that estimates could be made with reference to the pK_a values of model compounds such as II and III.

pK_a Values and Structure. Compound Ic differs from Ia by a chlorine substituent. The lesser basicity of Ic is as might have been expected. Methylation of the anilino nitrogen of Ia, converting it to Ie, does not noticeably change the pK_a values, which again is to be expected. However, replacement of the 2-amino group in the pyrimidine moiety of Ia by a piperidino group, forming Id, *decreased* the basicity by a significant amount. As a check on the authenticity of this observation, pK_a determinations were carried out on 2-piperidinopyrimidin-4-one.[3] The values were found to be 3.57 and 9.49 (±0.05) for the gain and the loss of a proton, respectively. The constants for the corresponding 2-amino derivative, isocytosine, have been reported as 4.01 and 9.42.[10]

(9) J. F. Bunnett and F. P. Olsen, Abstracts, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963, p. 41Q.
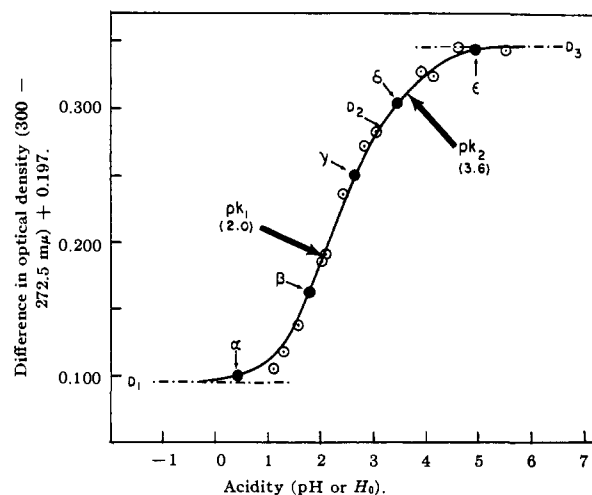

Figure 12. 2-Amino-5-(o-methylaminophenylthio)pyrimidin-4-one (Ie): (O.D.$_{300}$ − O.D.$_{272.5}$) vs. acidity (pH or H_0); solid circles, experimental points used in calculation of pK_1 and pK_2 from eq. 5; open circles, other experimental points; solid curve, calculated from eq. 5 using roots D_1 − D_5, pK_1 and pK_2 shown on this graph.

Related phenomena have been observed with certain 4-aminopyrimidin-6-ones, and are cited by Brown in a discussion of the ionization of pyrimidines[11] as "a curious phenomenon." Katritzky and Lagowski[12] have discussed the occasional base-weakening effects of the alkylation of amino groups in related heterocycles, where the results can be explained by the blocking of intramolecular hydrogen bonding which otherwise occurs, or by a change in the nature of the tautomeric structures.

Experimental

The methods employed for the spectrophotometric determinations were those of Roth and Schloemer.[3] The Brown University IBM 7070 computer was employed. Copies of the original data and calculations for the pK_a values of all the compounds described in this paper are on file in the Library, Burroughs Wellcome and Co., Tuckahoe, N. Y., and may be obtained on request from the librarian.

(10) P. A. Levene, L. W. Bass, and H. S. Simms, *J. Biol. Chem.,* 70, 229 (1926).

(11) D. J. Brown, "The Pyrimidines," John Wiley and Sons, Inc., New York, N. Y., 1962, pp. 470, 471.

(12) A. R. Katritzky and J. M. Lagowski in "Advances in Heterocyclic Chemistry," Vol. I, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, p. 327.