Summary

Three new N-[2-(3-indolyl)ethyl]thioamides (IIa∼c) and N-(methylthiocarbonyl) tryptophan methyl ester (III) were conveniently prepared according to our general procedure of synthesizing thioamides. The cyclodesulfurization of IIa∼c was successfully accomplished in high yield to the corresponding 1-substituted-3,4-dihydro-9*H*-pyrido[3,4-*b*]-indole derivatives (IVa∼c). In addition, III was cyclized to give a mixture of both 1-methyl-3,4-dihydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (Vb) and its methyl ester (Va) in excellent yield.

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115. **Eizo Hirai**: The Behavior of 4-Amino-5-carboxy-2-methylpyrimidine in Aqueous Solution.

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In the previous paper[1] it was found that the base strength of 4-amino-5-carboxy-2-methylpyrimidine (ACMP) was much lower than that estimated from the Hammett equation for the basic pKa value of 4,5-substituted 2-methylpyrimidines. Such deviation may result from any one of the following causes: (a) amino-imino tautomerism; (b) interaction between the carboxyl group and the neighboring amino group in the molecule; and (c) interaction between the carboxy group and the nuclear nitrogen atom in the molecule. However, it is impossible to attribute the deviation of ACMP from the Hammett equation to (a), because a similar deviation was found on the 4-dimethylamino analogue of ACMP, which necessarily possesses the amino structure. If it is caused by (b), considerable depression of base strength may be found on 2-aminonicotinic acid (ANA), but not so on 2-aminoisonicotinic acid (AINA). If it is caused by (c), depression may be found on both the base strengths of ANA and AINA. Hence the author examined the Hammett equations for the base strength of both series of 3- and 4-substituted 2-aminopyridines containing ANA and AINA prior to discussion of the behavior of ACMP in the present study.

Results and Discussion

The basic ionization constants of 3- and 4-substituted 2-aminopyridines are potentiometrically and spectrophotometrically determined. Since ANA and AINA are amphoteric, the acidic ionization constants also are spectrophotometrically determined. These constants are listed in Table I. As shown in Fig. 1, each Hammett equation for the basic pKa values of both series of 2-aminopyridines is represented by a linear relationship, with the exception of ANA and AINA. The meta- and para-substituent constants, σ_m and σ_p, are used as a measure of polar effect of the 3- and

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[*1] Fukushima-ku, Osaka (平井瑛三).

[1] S. Mizukami, E. Hirai: J. Org. Chem., **31**, 1199 (1966).

Table I. Ionization Constants

Substance	pKa (basic)		pKa (acidic)	
	Observed	Calcd.	Observed	Calcd.
2-Aminopyridine	6.86	—		
2-Amino-3-methylpyridine	7.20	7.37		
2-Amino-4-methylpyridine	7.38	7.71		
2-Aminonicotinic acid (ANA)	2.25	3.76	6.54	6.50
Methyl ester of ANA	4.78	4.82		
Ethyl ester of ANA	4.73	4.85		
1-Methyl betaine of ANA	2.08	—		
2-Aminoisonicotinic acid (AINA)	1.95	3.53	6.37	6.55
Methyl ester of AINA	5.21	4.95		
Ethyl ester of AINA	5.25	5.00		
1-Methyl betaine of AINA	1.91	—		
4-Amino-5-carboxy-2-methylpyrimidine (ACMP)	2.14		6.28	
Ethyl ester of ACMP	4.53			
1-Methyl betaine of ACMP	2.00			

4-substituent on the base strengths of these pyridines, respectively. This fact indicates that these 2-aminopyridines[2] exist largely as the 2-amino form in water and accept a proton at the nuclear nitrogen atom, as well as 4-aminopyrimidines.[1] Each the basic pKa values of ANA and AINA is much lower than that estimated from the corresponding Hammett equation, just as the values of AMPC and the 4-dimethylamino analogue are.

From another viewpoint, the polar effect of substituents on the base strength of multiple substituted pyridines is additive.[3] On the basis of this additive property, the basic pKa values of these 2-aminopyridines are calculated by the equation (1).

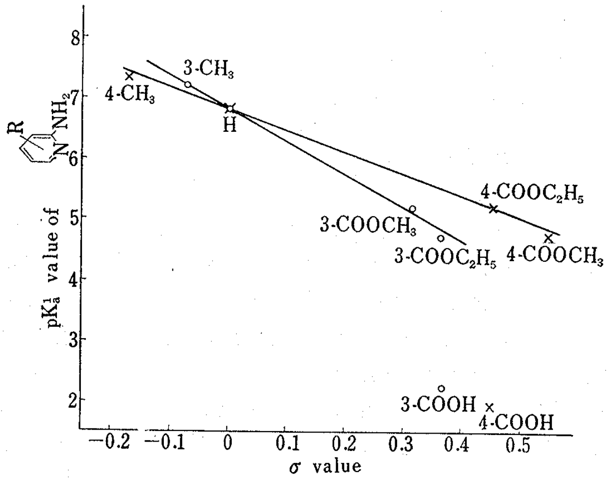
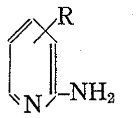
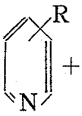


Fig. 1. Variation of the Basic pKa Values with σ Values for Pyridines

—○— 3-Substituted 2-aminopyridines
—×— 4-Substituted 2-aminopyridines

Basic pKa of 

=Basic pKa of  + Basic pKa of 2-aminopyridine − Basic pKa of pyridine (1)

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2) L.C. Anderson, N.V. Seeger: J. Am. Chem. Soc., **71**, 340 (1949); E.A. Steck, G.W. Ewing: *Ibid.*, **70**, 3397 (1948); S.J. Angyal, C.L. Angyal: J. Chem. Soc., **1952**, 1461; C.L. Angyal, R.L. Werner: *Ibid.*, **1952**, 2911; J.D.S. Goulden: *Ibid.*, **1952**, 2939.

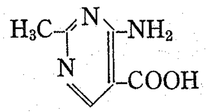
Although they reported that 2-aminopyridine exists largely in the amino form, none has described about the substituent effect on the amino-imino tautomerism of substituted 2-aminopyridines.

3) H.C. Brown, D.H. McDaniel, O. Häfliger: "Determination of Organic Structures by Physical Methods," R.A. Braude and F.C. Nachod, Eds., 612 (1955). Academic Press, New York.

The calculated pKa values are essentially identical with the measured ones, with the exception of ANA and AINA, as shown in Table I. The calculations for ANA and AINA are carried out by using the basic pKa values of nicotinic and isonicotinic acids, respectively, as the first term in the right side of the equation (1). Jaffé,[4] and Green and Tong[5] reported individually that nicotinic and isonicotinic acids exist largely as a zwitterion form rather than as an uncharged form in water. If so, the basic pKa values of these acids correspond to the acid strengths of their carboxy groups, while the acidic pKa values correspond to the base strengths of their pyridine nuclei. When the acidic pKa values of both acids are used in the first term in the equation (1), the calculated values are in good agreement with the acidic pKa values measured of ANA and AINA, respectively, as shown in Table I. This fact indicates that ANA and AINA exist largely in zwitterion form in water as well as nicotinic and isonicotinic acid.

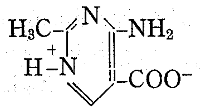
Although it is impossible to discuss likewise the behavior of ACMP in water because available data are very poor in the field of pyrimidine base, ACMP also may exist as the zwitterion in water.

In order to collect proof for the assumption, the ultraviolet absorption spectra of ACMP, ANA and AINA are compared with those of the corresponding ethyl ester and 1-methyl betaine. The ethyl ester and the 1-methyl betaine will structurally correspond to the uncharged form and the zwitterion, respectively. Hence Ia and Ib will be spectrophotochemically identical with II and III, respectively.



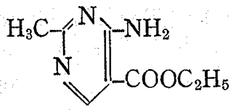
Ia

uncharged form

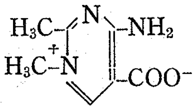


Ib

zwitterion



II



III

Because the acidic and basic pKa values are 6.28 and 2.14,[1] respectively, ACMP will exist almost completely as Ia and/or Ib in the solution of pH 4.2, which is the isoelectric point. The ultraviolet absorption spectrum of ACMP at the isoelectric point closely resembles that of the betaine at pH 7 rather than that of the ester at pH 7 as shown in Fig. 2. At pH 7 the betaine and the ester exist completely as the neutral forms which are shown by II and III, respectively. Similar results for ANA and AINA are shown in Fig. 3 and 4.

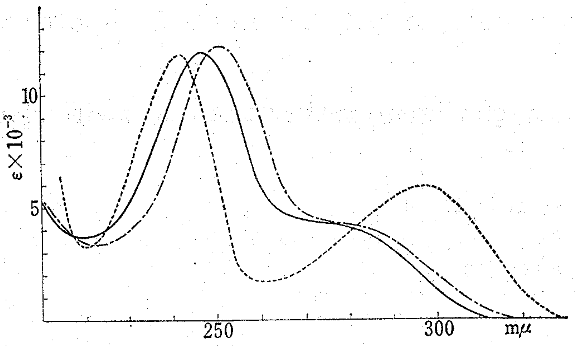


Fig. 2. Ultraviolet Absorption Spectra (in Water)

———— 4-Amino-5-carboxy-2-methylpyrimidine (ACMP) at pH 4.2
- - - - - Ethyl ester of ACMP at pH 7.0
—·—·— 1-Methyl betaine of ACMP at pH 7.0

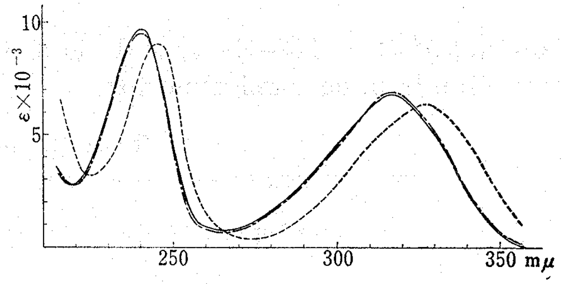


Fig. 3. Ultraviolet Absorption Spectra (in Water)

———— 2-Aminonicotinic acid (ANA) at pH 4.4
- - - - - Ethyl ester of ANA at pH 8.0
—·—·— 1-Methyl betaine of ANA at pH 7.0

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4) H.H. Jaffé: J. Am. Chem. Soc., **77**, 4445 (1955).
5) R.W. Green, H.K. Tong: *Ibid.*, **78**, 4896 (1956).

Further, each the basic pKa values of ACMP, ANA and AINA is in good agreement with that of the corresponding 1-methyl betaine as shown in Table I.

These results, although qualitative, indicate that ACMP, ANA and AINA exist predominantly in the zwitterion form in water.

A quantitative evaluation for zwitterion formation of these compounds is carried out by using the method derived by Edsall and Blanchard[6] to estimate an equilibrium constant, K_Z, between zwitterion and uncharged form of simple aliphatic amino acids. The K_Z value is represented by the equation (2),

$$K_Z=\frac{[\text{zwitterion}]}{[\text{uncharged form}]}\fallingdotseq\frac{K_a}{K_E}-1 \qquad (2)$$

where K_a and K_E are the basic ionization constants of an amino acid and its ester, respectively.

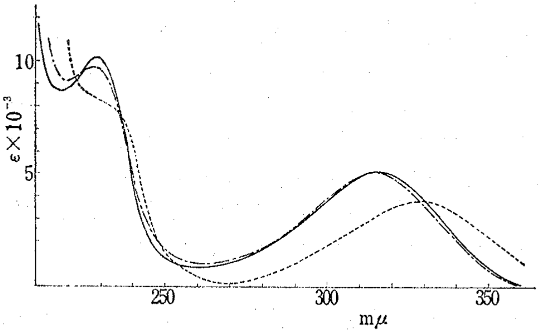

Fig. 4. Ultraviolet Absorption Spectra (in Water)

——— 2-Aminoisonicotinic acid (AINA) at pH 4.2
······ Ethyl ester of AINA at pH 7.0
– – – – 1-Methyl betaine of AINA at pH 7.0

The calculated K_Z values of ACMP, ANA and AINA are presented in Table II. These results confirm the impression gained from the absorption spectra that these compounds exist largely in zwitterion form in water.

Table II. The K_Z Values and Zwitterion Formation

Substance	log K_Z	Zwitterion (%)
ACMP	2.23	99.6
ANA	2.48	99.7
AINA	3.30	99.9

On the other hand, the infrared spectra of ACMP, ANA and AINA in Nujol are measured. These compounds show strong absorption for the carboxyl group at around 1700 cm^{-1}, as shown in Table III. Moreover, two broad absorption bands are found near 2400 and 1900 cm^{-1}. Yoshida and Asai[7] found that the similar bands of pyrazine- and pyridine-carboxylic acids were due to a hydrogen bonding between a hydrogen atom in carboxy group and a nuclear nitrogen atom. Therefore, it is assumed that ACMP, ANA and AINA in solid state form an uncharged polymer linked by hydrogen bonding of $N\cdots\cdots HO-\overset{\overset{O}{\|}}{C}-$ type (N is a nuclear nitrogen atom) rather than a zwitterion or a simple uncharged molecule.

Table III. Infrared Spectra in Nujol

Substance	Frequency (cm^{-1})			
	ν_{NH}	$\nu_{C=O}$		
ACMP	3250 3105	1670	2380	2030
ANA	3270	1708	about 2450	1985
AINA	3285	1695	〃 2350	1965

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[6] J. T. Edsall, M. H. Blanchard: *Ibid.*, **56**, 2337 (1933).

[7] S. Yoshida, M. Asai: This Bulletin, **7**, 162 (1959).

Experimental

Determination of Ionization Constants——The potentiometric determinations were carried out by the following way. A weighed quantity (about 5×10^{-4} g.-mole) of the compounds was dissolved in 100 ml. of CO_2-free water and titrated with the solution of *N*/10 HCl or NaOH (carbonate free) under passing N_2 gas. All pH measurements were made with a Yanagimoto potentiometric titrimeter, KY-6, equipped with a glass-calomel electrode pair. The ionization constants were calculated from the experimental points in the buffer region of each the titration curves according to the method described by Albert.[8]

The spectrophotometric method was used for the determination of the basic ionization constants of the very weak bases and the very slightly soluble bases. A $1\times10^{-4}M$ solution of the base was prepared in a buffer solution, and the variation of its absorbance with pH of buffer solution was measured at a selected wave length in a Hitachi spectrophotometer, EPU-2A. Britton and Robinson's buffer solutions[9] were suitable for the measurements. The ionization constants were calculated by the method of Albert.[8]

Materials——The following compounds had the melting points (or boiling point) and properties reported in the literature and were prepared according to the cited references: 2-Aminopyridine, m.p. 55~56°;[10] 2-Amino-3-methylpyridine, b.p. 102~104°(11 mm.);[11] 2-Amino-4-methylpyridine, m.p. 99~101°;[12] 2-Aminonicotinic acid, m.p. above 300°;[13] Methyl 2-aminonicotinate, m.p. 82~84°;[13] Ethyl 2-aminonicotinate, m.p. 94~95°;[14] 2-Aminoisonicotinic acid, m.p. 240°;[15] Methyl 2-aminoisonicotinate, m.p. 145~146°;[15] Ethyl 2-aminoisonicotinate, m.p. 121~123°;[15] 4-Amino-5-carboxy-2-methylpyrimidine, m.p. 267°[16] (decomp.); and 4-Amino-5-ethoxycarbonyl-2-methylpyrimidine, m.p. 121°.[17]

1-Methyl Betaine of 4-Amino-5-carboxy-2-methylpyrimidine——To the solution of 1 g. of 4-amino-5-ethoxycarbonyl-2-methylpyrimidine in 10 ml. of acetone was added 4 ml. of methyl iodide. The solution was heated under refluxing for 1 hr. on a steam bath. After cooling, a crystal separated. The crystal was dissolved in 10 ml. of 5% hydroiodic acid and heated for 1 hr. on a steam bath. After elimination of the iodide ion by adding a strong basic ion exchange resin (Amberlite IRA-400, OH-type), the reaction mixture was evaporated to dryness under reduced pressure. The residue was recrystallized from aqueous ethanol to give 0.6 g. of colorless crystals, m.p. 226~227°(decomp.). *Anal.* Calcd. for $C_7H_9O_2N_3\cdot\frac{1}{2}H_2O$: C, 47.72; H, 5.72; N, 23.85. Found: C, 47.63; H, 5.98; N, 24.13.

1-Methyl Betaine of 2-Aminonicotinic Acid——The betaine was synthesized from ethyl 2-aminonicotinate by a similar method to that of 1-methyl betaine of 4-amino-5-carboxy-2-methylpyrimidine, m.p. 269~271° (decomp.). *Anal.* Calcd. for $C_7H_8O_2N_2$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.36; H, 5.45; N, 18.66.

1-Methyl Betaine of 2-Aminoisonicotinic Acid——The betaine was prepared from ethyl 2-aminoisonicotinate by a similar method to preparation of both the betaines of 4-amino-5-carboxy-2-methylpyrimidine and 2-aminonicotinic acid.

The author expresses his gratitude to Dr. K. Takeda, Director of this Laboratory, and Dr. S. Mizukami for their helpful guidance and encouragement.

Summary

The behavior of 4-amino-5-carboxy-2-methylpyrimidine (ACMP) was compared with those of 2-aminoisonicotinic acid (ANA) and 2-aminoisonicotinic acid (AINA) in water. The behaviors of these compounds were investigated spectrophotometrically by comparison with those of the corresponding ethyl ester and 1-methyl betaine. The Edsall-Blanchard equation was used to evaluate the equilibrium constants, K_Z between zwitterion form and uncharged form in water. The evaluated K_Z values indicated that ACMP, ANA and AINA existed almost completely in the zwitterion form in water, respectively. This result was in good agreement with the spectrophotochemical observations.

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