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157. **Eizo Hirai**: The Behavior of 4-Amino-5-carboxy-2-methylpyrimidine in Glacial Acetic Acid and in Mixed Solvent of Isopropanol and Ethyleneglycol.

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The behavior of 4-amino-5-carboxy-2-methylpyrimidine (ACMP) in glacial acetic acid and the mixed solvent of isopropanol and ethyleneglycol was spectrophotometrically investigated by comparison with those of the ethyl ester and the 1-methyl betaine. The modified Edsall's equation was proposed to calculate potentiometrically the equilibrium constant (K_z value) between the zwitterion and the uncharged form of ACMP in these solvents. These results indicated that the zwitterion formation of ACMP was more repressed in these solvents than in water. The similar behavior was observed on the cases of 2-aminonicotinic acid (ANA) and 2-aminoisonicotinic acid (AINA) in these solvents. Further, the effect of the dielectric constant of solvent on the zwitterion formation of ACMP, ANA and AINA was spectrophotometrically examined in various alcohols and the mixed solvents of dioxane and water. The zwitterion formation was parallel with increasing of the dielectric constant of solvent.

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It was published in the previous paper[1] that 4-amino-5-carboxy-2-methylpyrimidine (ACMP), 2-aminonicotinic acid (ANA) and 2-aminoisonicotinic acid (AINA) exist predominantly as a zwitterion in water. However, there are but few investigations of a solvent effect on such zwitterion formation. Harris, *et al.*[2] and Metzler and Snell[3] found ultraviolet spectrophotometrically that the zwitterion formation of 3-hydroxypyridines was considerably repressed in ethanol and in dioxane-water mixture. Mizukami and the author reported in the previous paper[4] that by comparison of the half neutralization potentials (H.N.P. value) the basicity of ACMP was considerably enhanced in glacial acetic acid and in the mixed solvent of isopropanol and ethyleneglycol (1:1), particularly became as strong as that of the ethyl ester of ACMP in the former.

If such enhancement on the basicity of ACMP depends on only the equilibrium between the zwitterion and the uncharged form, it may be interpreted as repression of the zwitterion formation in the nonaqueous solvents as shown by the Edsall's equation[5] (1).

$$K_z = \frac{[\text{zwitterion}]}{[\text{uncharged form}]} \fallingdotseq \frac{K_a}{K_a^E} - 1 \tag{1}$$

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[1] E. Hirai: This Bulletin, **14**, 861 (1966).

[2] S.A. Harris, T.J. Webb, K. Folkers: J. Am. Chem. Soc., **62**, 3198 (1940).

[3] D.E. Metzler, E.E. Snell: *Ibid.*, **77**, 2431 (1955).

[4] S. Mizukami, E. Hirai: This Bulletin, **14**, 1321 (1966).

[5] J.T. Edsall, M.H. Blanchard: J. Am. Chem. Soc., **55**, 2337 (1933).

Here K_z is the equilibrium constant between zwitterion and uncharged form. K_a and K_a^E are the ionization constants of protonated ACMP and the ethyl ester, respectively.

This interpretation may be supported by the fact that the H. N. P. value of ACMP in glacial acetic acid was essentially identical with the one estimated from the Hammett's equation by using the meta substituent constant of COOH group.[4]

The present investigation is undertaken to clarify the behavior of ACMP in glacial acetic acid and in the mixed solvent of isopropanol and ethyleneglycol, and further to examine an effect of the dielectric constant of solvents on the zwitterion formation.

Moreover, the behaviors of ANA and AINA in these solvents are potentiometrically and spectrophotometrically investigated in this paper.

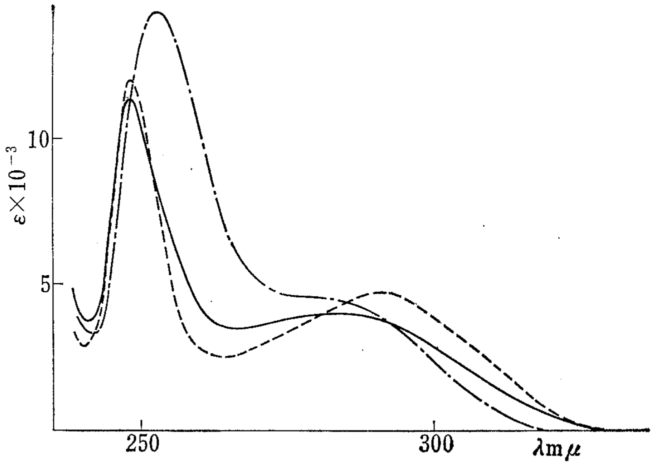


Fig. 1. Ultraviolet Absorption Spectra of 4-Amino-5-carboxy-2-methylpyrimidine, the Ethyl Ester and the 1-Methyl Betaine in Glacial Acetic Acid

——— 4-Amino-5-carboxy-2-methylpyrimidine
·········· Ethyl Ester
– – – – 1-Methyl Betaine

Results and Discussion

Behavior of ACMP in Glacial Acetic Acid

The ultraviolet absorption spectra of the uncharged form and the zwitterion of ACMP essentially must be identical with those of the ethyl ester and the 1-methyl betaine of ACMP, respectively. The spectra of ACMP, its ethyl ester, and its 1-methyl betaine in glacial acetic acid are shown in Fig. 1.

It is necessary to consider following equilibria for ACMP, the ester and the betaine, respectively, in this solvent.

$$\begin{tikzcd}
\underset{\text{Ia}}{\mathrm{NRCOOH + HAc}} \arrow[rr, shift left] \arrow[dr, shift left] & & \underset{\text{Ib}}{\mathrm{H\overset{+}{N}RCOO^- + HAc}} \arrow[ll, shift left] \arrow[dl, shift left] \\
& \underset{\text{II}}{\mathrm{H\overset{+}{N}RCOOH\cdot Ac^-}} \arrow[ul, shift left] \arrow[ur, shift left] \arrow[d, shift left] & \\
& \underset{\text{III}}{\mathrm{H\overset{+}{N}RCOOH + Ac^-}} \arrow[u, shift left] &
\end{tikzcd}$$

$$\underset{\text{IV}}{\mathrm{NRCOOEt + HAc}} \rightleftharpoons \underset{\text{V}}{\mathrm{H\overset{+}{N}RCOOEt\cdot Ac^-}} \rightleftharpoons \underset{\text{VI}}{\mathrm{H\overset{+}{N}RCOOEt + Ac^-}}$$

$$\underset{\text{VII}}{\mathrm{H_3\overset{+}{C}\cdot NRCOO^- + HAc}} \rightleftharpoons \underset{\text{VIII}}{\mathrm{H_3\overset{+}{C}\cdot NRCOOH\cdot Ac^-}} \rightleftharpoons \underset{\text{IX}}{\mathrm{H_3\overset{+}{C}\cdot NRCOOH + Ac^-}}$$

When sodium acetate is added to each solution of these compounds, it may be expected to repress dissociation of the ion-pairs, II, V and VIII. However, each spectra of these compounds is not affected by adding sodium acetate. Further, the ultraviolet spectra of the protonated cations of these compounds are essentially identical as found from those of their perchlorates. These spectral results are listed in Table I. Accordingly, the difference in the spectra between the ester and the betaine must result from difference in spectra between Ⅳ and Ⅶ remaining in the solutions. The experimental result shows that the spectrum of ACMP resembles that of the ester rather than the betaine in glacial acetic acid as shown in Fig. 1. This fact, therefore, indicates that the zwitterion formation of ACMP is considerably repressed in this solvent.

Table I. Data of Ultraviolet Absorption Spectra in Acetic Acid

Solvent / Substance	Acetic acid		$0.1N$ $HClO_4$ (in AcOH)		$0.01M$ AcONa (in AcOH)	
	λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε
4-Amino-5-carboxy-2-methylpyrimidine	249	4.05	248	4.13	249	4.03
	285	3.62	280	3.63	285	3.64
Ethyl ester	249	4.07	248	4.13	249	4.06
	293	3.66	280	3.63	293	3.66
1-Methyl betaine	252	4.17	251	4.20	252	4.16
	277[a]	3.69	287	3.66	277[a]	3.68
2-Aminonicotinic acid	250	3.94	249	3.91	250	3.94
	333	3.82	326	3.90	333	3.83
Ethyl ester	250	3.91	249	3.89	250	3.92
	332	3.86	326	3.89	332	3.83
1-Methyl betaine	320	3.89	326	3.87	320	3.90
2-Aminoisonicotinic acid	339	3.68	334	3.73	339	3.67
Ethyl ester	339	3.67	334	3.73	339	3.67
1-Methyl betaine	328	3.61	331	3.62	328	3.61

a) shoulder

In order to determine quantitatively zwitterion formation in this solvent, although a rough approximation, the equation (3) is derived from the equations (1) and (2). The equation (2) indicates a linear relationship between the p*K*a (H_2O) values of 5-substituted 4-amino-2-methylpyrimidines and their H.N.P. values in a given solvent.[4]

$$\text{H.N.P. value} = \alpha \cdot \mathrm{p}K\mathrm{a} + \beta \qquad (2)$$

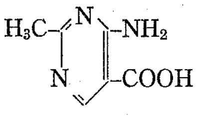
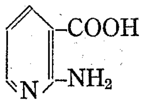
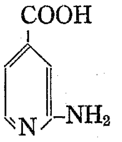
$$K_z \doteqdot 10^{-\left(\frac{\text{H.N.P.}^{\text{I}} - \text{H.N.P.}^{\text{E}}}{\alpha}\right)} - 1 \qquad (3)$$

Here H.N.P.^{I} and H.N.P.^{E} are the half neutralization potentials of ACMP and its ethyl ester, respectively. The calculated K_z value by the equation (3) is shown in Table Ⅱ. The result also clearly indicates that ACMP exists more as the uncharged form than as the zwitterion in this solvent.

Behavior of ACMP in the Mixed Solvent of Isopropanol and Ethyleneglycol

Since ACMP is assumed to exist as the uncharged form in solid state,[1] the dissociation of the COOH group in the molecule may be at first required to form the

Table II. Equilibrium Constant between Zwitterion and Uncharged Form of ACMP, ANA and AINA in Glacial Acetic Acid and Isopropanol-Ethyleneglycol (1:1)

Substance		Solvent	
		Glac. acetic acid	Isopropanol-Ethyleneglycol
	H. N. P.^{I} (mV)	400$^{a)}$	232$^{a)}$
	H. N. P.^{E} (mV)	398$^{a)}$	206$^{a)}$
	α	−17.1$^{a)}$	−66.7$^{a)}$
	K_z	0.309	1.453
	Zwitterion (%)	23.6	59.2
	H. N. P.^{I} (mV)	422	191
	H. N. P.^{E} (mV)	420	220
	α	−10.2$^{b)}$	−59.2$^{b)}$
	K_z	0.570	2.089
	Zwitterion (%)	36.3	67.7
	H. N. P.^{I} (mV)	439	
	H. N. P.^{E} (mV)	438	
	α	−7.7$^{b)}$	
	K_z	0.348	
	Zwitterion (%)	25.8	

a) Ref. 2).
b) Calculated from the linear relationships in Fig. 3.

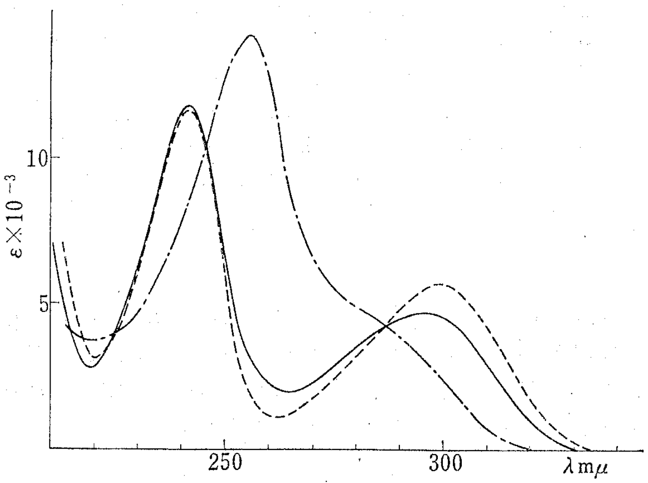


Fig. 2. Ultraviolet Absorption Spectra of 4-Amino-5-carboxy-2-methylpyrimidine, the Ethyl Ester and the 1-Methyl Betaine in Isopropanol

——— 4-Amino-5-carboxy-2-methylpyrimidine
-------- Ethyl Ester
– – – – 1-Methyl Betaine

zwitterion in a given solvent.[*2] Since isopropanol is a solvent of low dielectric constant, it may be expected that the dissociation of the COOH group of ACMP is so prevented in the solvent that the zwitterion formation is considerably repressed.[6)]

The ultraviolet absorption spectrum of ACMP in isopropanol is compared with those of the ethyl ester and the 1-methyl betaine in Fig. 2.

Since alcohols can serve as electron donors or electron acceptors, if ACMP exist in the uncharged form, they must form solvate bonds with ACMP, probably as

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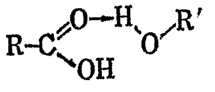
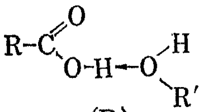
[*2] The dissociation of the COOH group and the protonation to the ring nitrogen atom (1-position) in the molecule may be concurrently required to the zwitterion formation of ACMP in the protic solvents. However, the protonation to the uncharged form of ACMP is negligible in the alcoholic solvents used as shown by comparison of the ultraviolet spectra of the ethyl ester of ACMP in the solvents with that in the aqueous solution (pH 7), Table III and V. Therefore, it may be assumed that the zwitterion of ACMP in the alcoholic solvents is formed by the dissociation of the COOH group, and then by the protonation to the ring nitrogen atom because of increasing of the basicity by the electron-releasing effect of the COO$^-$ group.

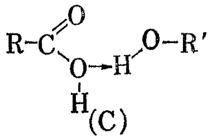
6) H. E. Ungnade, R. W. Lamb: J. Am. Chem. Soc., **74**, 3789 (1952).



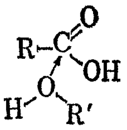
(A)



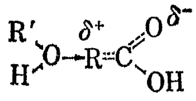
(B)



(C)



(D)



(E)

Each solvation will exert electronic effect on the resonance system in ACMP. All of these solvation except the type (B) are also formed between alcohols and the ethyl ester of ACMP. The solvation of type (B), however, does not appreciably affect the spectrum as pointed out by Ungnade and Lamb.6) Therefore, the difference in the spectra between ACMP and the ethyl ester in alcohols must be interpreted from the extent of the zwitterion formation of the former.

The spectrum of ACMP closely resembles that of the ethyl ester in isopropanol. This observation leads to a conclusion that the zwitterion formation of ACMP is almost completely repressed in this solvent.

Table III. Ultraviolet Absorption Spectra in Isopropanol-Ethyleneglycol Mixed Solvents

Solvent / Substance		Isopropanolb)		Isopropanol–Ethyleneglycol (4:1)		Isopropanol–Ethyleneglycol (1:1)		Ethyleneglycolc)	
		λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε
4-Amino-5-carboxy-2-methylpyrimidine		242	4.06	244	4.04	244.5	4.06	246	4.08
		296	3.68	291	3.60	287	3.62	284	3.62
	Ethyl ester	242	4.06	242	4.06	242.5	4.09	242.5	4.12
		299.5	3.74	300	3.74	300	3.75	300	3.76
	1-Methyl betaine	255.5	4.15	255	4.15	254	4.15	254	4.16
		280a)	3.72	280a)	3.69	280a)	3.69	280a)	3.69
2-Aminonicotinic acid		246.5	3.96	246	3.95	245.5	3.99	245.5	4.01
		330.5	3.79	327.5	3.76	324	3.77	321.5	3.75
	Ethyl ester	248	3.99	248	3.99	248.5	4.04	248.5	4.02
		332.5	3.85	334.5	3.85	334.5	3.87	334.5	3.85
	1-Methyl betaine	241	3.95	241	3.94	241	3.94	241.5	3.96
		318	3.82	318	3.85	319	3.86	319.5	3.86
2-Aminoisonicotinic acid		236	—	235	3.88	234.5	3.90	234	3.94
		329	—	322.5	3.51	321	3.52	319.5	3.52
	Ethyl ester	241	3.97	240.5	4.01	240.5	3.98	240	4.01
		337	3.54	337	3.54	337	3.54	337	3.54
	1-Methyl betaine	233	3.89	232	3.90	232	3.90	230	3.90
		316	3.66	316.5	3.65	318	3.65	319	3.62

a) shoulder b) dielectric constant 18.3 c) dielectric constant 37.7

On adding ethyleneglycol to each solution of ACMP, the ethyl ester and the 1-methyl betaine in isopropanol, changes of these spectra are observed. As shown in Table III, the spectrum of ACMP gradually approaches to that of the betaine from the ester with increasing of ratio of amounts of ethyleneglycol to isopropanol, but both spectra of the betaine and the ester are independent of the ratio of the components in the mixed solvent. These results, therefore, evidently indicate that the zwitterion formation of ACMP increase with increasing of the ratio of ethyleneglycol to isopropanol in the mixed solvent.

The K_z value of ACMP in the mixed solvent (1:1) is calculated by the equation (3) and is shown in Table II. This result is in good agreement with the above observation.

Behaviors of ANA and AINA in Glacial Acetic Acid and the Mixed Solvent of Isopropanol and Ethyleneglycol

The H.N.P. values of 3- and 4-substituted 2-aminopyridines in glacial acetic acid and the mixed solvent of isopropanol and ethyleneglycol (1:1) are determined from the potentiometric titration curves with perchloric acid. Only AINA cannot be titrated in isopropanol-ethyleneglycol mixed solvent because it is slightly soluble. The relationships between the H.N.P. values and their p*K*a (H_2O)[1] are shown in Fig. 3. Further, the Hammett's equations for the H.N.P. values are examined by using the meta and para substituent constants for 3- and 4-substituents, respectively, as shown in Fig. 4.

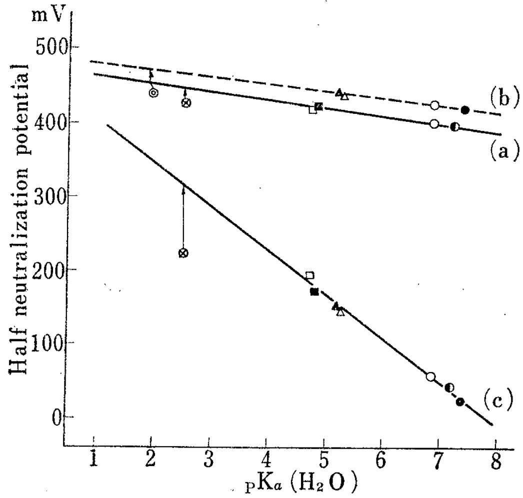


Fig. 3. Relationship between p*K*a (H_2O) of 3- and 4-Substituted 2-Aminopyridines and their Half Neutralization Potentials in Glacial Acetic Acid and Isopropanol-Ethyleneglycol

(a) and (b); Glacial acetic acid
(c); Isopropanol-Ethyleneglycol
Substituent: ○; H, ◑; 3-CH_3, ●; 4-CH_3, □; 3-$COOC_2H_5$, ■; 3-$COOCH_3$, ⊗; 3-COOH, △; 4-$COOC_2H_5$, ▲; 4-$COOCH_3$, ◎; 4-COOH

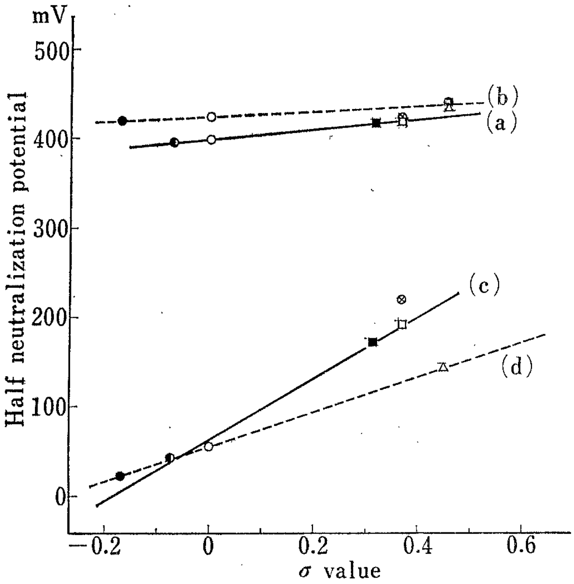


Fig. 4. Relationship between Hammett's σ and Half Neutralization Potentials in Glacial Acetic Acid and Isopropanol-Ethyleneglycol

(a) and (b); Glacial Acetic acid (c) and (d); Isopropanol-Ethyleneglycol
Substituent: ○; H, ◑; 3-CH_3; ●; 4-CH_3, □; 3-$COOC_2H_5$, ■; 3-$COOCH_3$, ⊗; 3-COOH, △; 4-$COOC_2H_5$, ◎; 4-COOH

Only the H.N.P. values of the 3- and 4-carboxy derivatives, ANA and AINA, deviate considerably from the linear relationships against the p*K*a (H_2O). However, these H.N.P. values in glacial acetic acid do not deviate from the Hammett's equations when the meta and para substituent constants of COOH group are used as the parameters of both the compounds, respectively. The behavior of ANA in both the solvents and that of AINA in glacial acetic acid closely resemble that of ACMP.[4]

On the other hand, the ultraviolet absorption spectra of ANA and AINA in glacial acetic acid and the mixed solvent of isopropanol and ethyleneglycol are compared with those of the corresponding ethyl ester and 1-methyl betaine in Table I and Table III. These data indicate that the behaviors of both compounds in these solvents are

Table IV. Ultraviolet Absorption Spectra in Methanol and Ethanol

Solvent	Methanol		Ethanol	
Dielectric const.	32.6		24.3	
Substance	λmax (mμ)	log ε	λmax (mμ)	log ε
4-Amino-5-carboxy-2-methylpyrimidine	242	4.03	242	4.01
	289	3.67	290.5	3.63
Ethyl ester	242.5	4.07	242	4.09
	299	3.74	299	3.74
1-Methyl betaine	254	4.09	254.5	4.00
	280a)	3.64	278a)	3.57
2-Aminonicotinic acid	244.5	4.02	246	3.99
	324.5	3.78	328	3.78
Ethyl ester	247.5	4.00	247.5	3.99
	315.5	3.84	332.5	3.83
1-Methyl betaine	240	3.96	241	3.95
	318	3.85	318	3.87
2-Aminoisonicotinic acid	234.5	—	237	—
	320	—	327.5	—
Ethyl ester	240	4.00	239.5	3.96
	335	3.54	335.5	3.45
1-Methyl betaine	231	3.86	232.5	3.86
	315.5	3.62	316	3.64

a) shoulder

Table V. Ultraviolet Absorption Spectra in Water-Dioxane Mixed Solvents

Solvent	Waterb)		Water-dioxane ≑(17:8) wt/wt		Water-dioxane ≑(10:15) wt/wt		Water-dioxane ≑(5:20) wt/wt	
Dielectric const.	78.5		51.9		25.9		10.7	
Substance	λmax (mμ)	log ε	λmax (mμ)	log ε	λmax (mμ)	log ε	λmax (mμ)	log ε
4-Amino-5-carboxy-2-methylpyrimidine	247	4.08	246	4.06	245.5	4.06	243.5	4.00
	275a)	3.64	277	3.62	277.5	3.61	289	3.59
Ethyl ester	241～242	4.07	242	4.08	242	4.07	242.5	4.04
	297±1	3.76	299	3.78	299	3.76	299	3.75
1-Methyl betaine	251～252	4.10	252.5	4.12	254	4.12	254.5	4.11
	277a)	3.64	277a)	3.67	276a)	3.69	278a)	3.69
2-Aminonicotinic acid	240	3.99	241.5	3.96	243.5	3.96	247	3.99
	317～318	3.83	318	3.78	321.5	3.76	330	3.76
Ethyl ester	245～246	3.95	247.5	3.97	248	3.99	248.5	3.99
	327±1	3.79	332	3.82	333.5	3.84	334	3.84
1-Methyl betaine	240	3.89	240.5	3.81	240.5	3.81	241	3.86
	317～318	3.83	317.5	3.75	318	3.74	318	3.74
2-Aminoisonicotinic acid	228	4.01	230	3.99	231	3.98	237.5	3.97
	316	3.72	317.5	3.66	318.5	3.65	325	3.64
Ethyl ester	230a)	3.91	238	3.98	240	3.99	240.5	3.99
	329±1	3.56	333	3.50	336.5	3.53	336.5	3.54
1-Methyl betaine	227	3.97	229	3.90	229.5	3.90	230.5	3.89
	316	3.71	317	3.62	317	3.62	316	3.63

a) shoulder b) Ref. 1)

similar to that of ACMP. Therefore, it is evident that the zwitterion formation of ANA and AINA is much more repressed in glacial acetic acid and the mixed solvent of isopropanol and ethyleneglycol than in water as well as ACMP.

The K_z values of ANA and AINA in glacial acetic acid and the mixed solvent of isopropanol and ethyleneglycol (1:1) are calculated by the equation (3) and are listed in Table II. These results are in good agreement with the spectroscopic observations.

Effect of Dielectric Constant of Solvent on Zwitterion Formation

It is presumed that the dielectric constant will increase on adding ethyleneglycol to isopropanol. Thus the zwitterion formation of ACMP, ANA and AINA may be correlative to the dielectric constant of solvent.

In Table III, IV and V the spectra of these compounds are compared with those of the corresponding ethyl ester and 1-methyl betaine in various alcohols and in the mixed solvents of dioxane and water. The dielectric constants of the latters were measured by Åkerlöf and Short.[7] Each spectra of ACMP, ANA and AINA changes from the ester type to the betaine type with increasing of the dielectric constant of both series of the solvents. However, the spectra of the betaines and the esters are little affected by the kind and the components of solvents. Therefore, it is evident that the zwitterion formation of ACMP, ANA and AINA is repressed with decreasing of the dielectric constant of solvent.

On the other hand, although both the dielectric constants of ethanol and the mixed solvent of dioxane and water (15:10) are practically identical, each spectra of ACMP, ANA and AINA much more resembles that of the corresponding betaine in the latter solvent than in the former solvent. Moreover, each spectra of ACMP, ANA and AINA in methanol is essentially identical with that in 20% aqueous dioxane, although the dielectric constant of the latter is much lower than that of the former. These results indicate that any other factor, in addition to the dielectric constant of solvent, may contribute to the zwitterion formation. According to the Edsall's equation,[5] the equilibrium constant between the zwitterion and the uncharged form is determined by ratio of the acidity of the protonated cations of both the forms. On the cases of ACMP, ANA and AINA, the p*K*a of the zwitterion is the acidity of the COOH group, but the p*K*a value of the uncharged form is the acidity of the protonated nuclear nitrogen. Kilpatrick[8] pointed out that solvent effect of ethanol on the acidity of COOH type considerably differs from that of ammonium ion type. Therefore, such solvent effect may be another factor which contributes to the zwitterion formation.

Experimental

Ultraviolet Absorption Spectra——Accurately weighed amounts of ACMP, ANA, AINA, their ethyl esters and their 1-methyl betaines were dissolved in the media listed in Table I, III and IV. Each concentration of the solutions required for measurements was about 15 μg. per 1 ml. These measurements were made with a Hitachi spectrophotometer, EPU-2A.

Potentiometric Titrations——Approximately 1×10^{-3} g.-mole of the samples, 3- and 4-substituted 2-aminopyridines, was accurately weighed and dissolved in 100 ml. of the solvents studied (in glacial acetic acid, 1×10^{-4} g.-mole of 4-substituted 2-aminopyridines was weighed). 0.1*N* Perchloric acid was used as the titrant. The solvent of titrant was the same as that of the samples. The potentiometric titrations were made with a Yanagimoto potentiometric titrimeter, KY-6, equipped with glass and calomel electrodes. The detailed manner was described in the previous paper.[4]

Materials——4-Amino-5-carboxy-2-methylpyrimidine (ACMP), 2-aminonicotinic acid (ANA), 2-aminoisonicotinic acid (AINA), their 1-methyl betaines, their ethyl esters and the methyl esters of ANA and AINA were prepared by the methods in the previous paper.[1] 2-Aminopyridine, 2-amino-3-methylpyridine

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7) G. Åkerlöf, O.A. Short: J. Am. Chem. Soc., **58**, 1241 (1936).

8) M. Kilpatrick: Chem. Revs., **30**, 159 (1942).

and 2-amino-4-methylpyridine, reagent grade, were obtained from Kantō Chemical Co., Inc., Japan.

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