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140. Kozo Nagano, Hirokazu Tsukahara, Hisashi Kinoshita, and Zenzo Tamura : Metal Complexes of Isonicotinoylhydrazine and Related Compounds. II.*1 Acid Dissociation Constants and Ultraviolet Absorption Spectra of Isonicotinoylhydrazine and Related Compounds.

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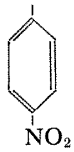
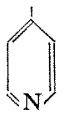
In the course of determining consecutive formation constants of metal complexes of isonicotinoylhydrazine (INH) and related compounds by means of pH titration, acid dissociation constants of them were determined, while, in order to get some informations about their structures in aqueous media of various pH, the ultraviolet absorption spectra were measured.

As the related compounds of INH, the following eight compounds were selected. Their abbreviated names, structural formulae and relative antituberculous activities are listed in Table I. INH, nicotinoylhydrazine (NH), picolinoylhydrazine (PH), benzoylhydrazine (BH) and *p*-nitrobenzoylhydrazine (PNBH) are slightly different from each other in the electronic character of their aromatic rings. Isonicotinamide (INA) is lack of the terminal amino group of INH. 1-Isonicotinoyl-1-methylhydrazine (N-Me-INH) and

Table I. Abbreviated Names, Structural Formulae and Relative Antituberculous Activites of INH and Related Compounds

Abbred. name	INH	NH	PH	BH
Structural formula				
Antituberc.	1	1/1600[1]	1/40[1]	1/1000[2]
Strain of *M. tuberc.*	H37Rv BCG	H37Rv	H37Rv	H37Rv

Abbred. name	PNBH	INA	N-Me-INH	N′-Me-INH
Atructural formula				
Antituberc.	1/1000[3]	<1/10000[4]	1/10000[5]	1/50[6]
Strain of *M. tuberc.*	H37Rv	H37Rv	H37Rv	BCG

*1 Part I : This Bulletin, **11**, 793 (1963).

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1) M. Ishikawa, S. Kishimoto, K. Tokuyama : Shionogi Kenkyujo Ho, **3**, 4 (1953).
2) Z. Horii, Y. Murakami, Y. Tamura, H. Uchida, Y. Yamamura, K. Miki, M. Kato : Yakugaku Zasshi, **76**, 1319 (1956).
3) M. Caesen, P. Van Dijck, H. Vanderhaeghe : J. Pharm. Pharmacol., **6**, 127 (1953).
4) E. M. Bavin, D. J. Drain, M. Seiler, D. E. Seymour : *Ibid.*, **4**, 844 (1952).
5) J. Cymerman-Craig, S. D. Rubbo, D. Willis, J. Edgar : Nature, **176**, 34 (1955).
6) J. Cymerman-Craig, D. Willis : J. Chem. Soc., **1955**, 4315.

1-isonicotinoyl-2-methylhydrazine (N'-Me-INH) possess a methyl group in the hydrazine of INH. Complete analysis of the pH titration data for these compounds will suggest the contribution of each group to the formation of the complexes.

Experimental

Materials——INH. Sumifon powder (Sumitomo Chem. Co.) was used without further purification, m.p. 163°.

NH was prepared and recrystallized from dil. EtOH,[7] m.p. 159°

PH was prepared and recrystallized from EtOH,[8] m.p. 102°

BH was prepared and recrystallized from H_2O,[9] m.p. 112.5°

PNBH was prepared and recrystallized from H_2O,[10] m.p. 210°

INA was prepared and recrystallized from EtOH,[11] m.p. 156°

N-Me-INH·2HCl was prepared.[6] N-Me-INH was obtained from N-Me-INH·2HCl and $(Dowex\text{-}1)^+$-HCO_3^-, and recrystallized from abs. EtOH. Colorless needles., m.p. 96.5~97.5°. *Anal.* Calcd. for $C_7H_9ON_3$: C, 55.61; H, 6.00; N, 27.80. Found : C, 55.66; H, 5.71; N, 27.45.

N'-Me-INH was prepared and recrystallized from abs. EtOH,[6] m.p. 77~78°.

Apparatus and Solutions——An apparatus of pH titration is shown schematically in Fig. 1. pH measurements were made with a Toa Denpa pH meter, Model HM-5, a Hokushin potentiometer, Model 301, and an ammeter of ±50 μA., and by adjusting an amplified current to zero.

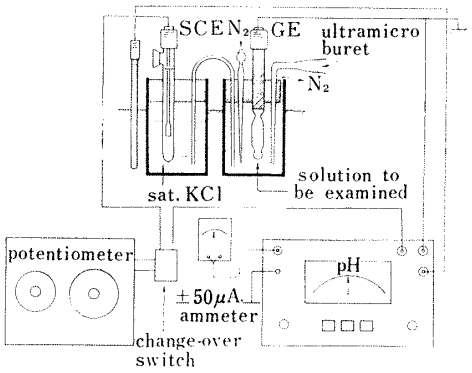


Fig. 1.

A Toa Denpa glass electrode, HG-2005, was used since its response to pH was rapid. By the use of a hydrogen electrode, the calibration curve (EMF *vs.* pH) of the glass electrode was confirmed to be a straight line over the range from pH 1.5 to 12.5 under the condition of ionic strength 1.0 (KNO_3). A Toa Denpa saturated calomel electrode, HC-205-2R, was used as a reference electrode. In order to keep junction potentials constant throughout the titration, the S.C.E. was dipped into a saturated KCl solution, and connected through a flexible saturated KCl salt bridge to the solution to be examined. A Toa Denpa automatic temperature-compensator, HR-105, was dipped into a constant temperature water bath held at 25.0±0.1°. The water bath and the earth terminal of pH meter were grounded. The EMF *vs.* pH calibration line was obtained by the method of least squares from the following 5 pH standards (Toa Denpa Co.): an oxalate buffer, pH 1.68; a phthalate buffer, pH 4.01; a phosphate buffer, pH 6.86; a borate buffer. pH 9.18; and a carbonate buffer, pH 10.02. The millivolt values for the solution to be examined were converted to pH according to the calibration line. This method is superior to the direct reading of pH with two point corrections in reproducibility over a wide pH range. In any given experiment pH could be estimated to 0.01.

Ionic strength of solutions were made to 1.0 by adding KNO_3. 1.0*N* NaOH free from CO_2 was prepared from 50% aqueous solution of reagent grade NaOH and was kept in a bottle of polyethylene shown in Fig. 2. The concentration of a compound was made to $2\times10^{-2}M$, 20 times as high as ordinary methods, in order to determine low pKa (1~2) and high pKa (12~13). The solution involved $4\times10^{-2}N$ HNO_3 at the beginning of titration. Nitrogen gas was passed through during titration in order to

7) T. Curtius, E. Mohr : Ber., **31**, 2493 (1898).
8) H. Meyer, J. Mally : Monatsch., **33**, 395 (1912).
9) G. Struve : J. prak. Chem., (2) **50**, 295 (1894).
10) T. Curtius, H. Melsbach : *Ibid.*, (2) **81**, 525 (1910).
11) R. Camps : Arch. Pharm., **240**, 361 (1902).

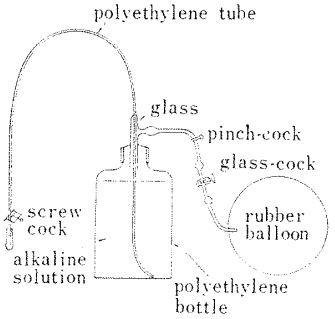


Fig. 2.

exclude CO_2 from the solution. 50 ml. of a solution was prepared for each compound, 10 ml. of which was used for one titration; and a Gilmont ultramicro buret with a scale of 1/1000 ml. and a total volume of 1.0 ml. (Emil & Greiner Co.) was used for the purpose.

The UV spectra were obtained with a Hitachi spectrophotometer, Type EPU-2A, and a Hitachi recording spectrophotometer, Type EPS-2. In all measurements of UV spectra, the concentration of the solution was $10^{-4}M$ and the cell thickness was 1.0 cm. Ionic strength of the solution was maintained 1.0 by the addition of KCl. The pH of the solution was measured before the spectral measurement with a Toa Denpa pH meter, Model HM-5, a glass electrode, HG-2005, and a reference electrode, HC-205-2R.

Treatment of Data ——pH Values were converted to hydrogen ion activities according to the following definition.

$$a_H = 10^{-pH} \tag{1}$$

When c_H is used as a hydrogen ion concentration in term of normality N, an activity coefficient of hydrogen ion is symbolized by f_H and defined by

$$a_H = f_H c_H \tag{2}$$

In alkaline region, a hydroxyl ion concentration c_{OH} is predominant over c_H and there is the following relation between c_{OH} and a_H,

$$Kw = a_H c_{OH} \tag{3}$$

where Kw is called the apparent dissociation product of H_2O. Before determining acid dissociation constants, $4\times10^{-2}N$ HNO_3 containing $1.0M$ KNO_3 was titrated several times with $1.0N$ NaOH, and f_H and Kw were obtained.

$$f_H = 0.94\pm0.02; \qquad Kw = (1.45\pm0.13)\times10^{-14} \tag{4}$$

If neutral molecules of all eight compounds are represented by HZ, acid dissociation constants, Ka_1, Ka_2 and Ka_3 can be defined according to the following equations.

$$Ka_1 = \frac{a_H[H_2Z]}{[H_3Z]}, \qquad Ka_2 = \frac{a_H[HZ]}{[H_2Z]}, \qquad Ka_3 = \frac{a_H[Z]}{[HZ]} \tag{5}$$

Here [ ] denotes a concentration of each ionic species in term of molarity M, and an electric charge of each ionic species is neglected. A formula H_jZ represents that $j-1$ protons are attached to the molecule, but does not tell about to what atoms they are attached in the molecule. Some of these compounds cannot take a form of H_3Z or Z. For instance, BH, PNBH and INA have not the first acid dissociation constant ($Ka_1=\infty$), and, as the neutral molecule of N-Me-INH cannot release proton any more, the third acid dissociation constant of it does not exist ($Ka_3=0$). But the above representation is convenient because, in most cases, the same symbol of Ka_j will be related to the corresponding structure. Besides, it should be noted that equilibrium constants, Ka_1, Ka_2 and Ka_3, are not thermodynamical but activity-concentration constants defined for convenience's sake.

In every point on titration curves the following two relations are valid, namely, that a total compound concentration $[Z]_T$ is equal to the sum of concentrations of all ionic species, and that a total proton concentration $[H]_T$ is equal to the sum of proton concentrations of all ionic species. According to Schwarzenbach,[12] the two relations are described as follows.

12) G. Schwarzenbach: Helv. Chim. Acta, 33, 947 (1950).

$$[Z]_T=\sum_{j=0}^{3}[H_jZ]=[H_3Z]+[H_2Z]+[HZ]+[Z] \quad (6)$$

$$[H]_T=c_H-c_{OH}+\sum_{j=0}^{3}j\cdot[H_jZ]=c_H-c_{OH}+3[H_3Z]+2[H_2Z]+[HZ] \quad (7)$$

When the number of moles of NaOH titrated per one mole of a compound is represented by a, the following relation holds.

$$[H]_T=[Z]_T(3-a) \quad (8)$$

Here,

$$g=3-a+\frac{c_{OH}-c_H}{[Z]_T} \quad (9)$$

is introduced, and the following equation is obtained from (7), (8) and (9).

$$[Z]_Tg=\sum_{j=0}^{3}j\cdot[H_jZ]=3[H_3Z]+2[H_2Z]+[HZ] \quad (10)$$

From (5), (6) and (10), the final equation is derived as

$$g+(g-1)a_H(Ka_3)^{-1}+(g-2)a_H^2(Ka_2Ka_3)^{-1}+(g-3)a_H^3(Ka_1Ka_2Ka_3)^{-1}=0 \quad (11)$$

For BH, PNBH and INA, $(Ka_1)^{-1}=0$. From the titration curves of them, $(Ka_2)^{-1}<10^5$ and $(Ka_3)^{-1}>10^{10}$. (11) is, therefore, splitted into two equations. In acidic region,

$$(g-1)+(g-2)a_H(Ka_2)^{-1}=0 \quad (11\text{-}1)$$

and, in alkaline region,

$$g+(g-1)a_H(Ka_3)^{-1}=0 \quad (11\text{-}2)$$

For INH, NH, PH, N-Me-INH and N′-Me-INH, $(Ka_1)^{-1}\sim(Ka_2)^{-1}<10^5$ and $(Ka_3)^{-1}>10^{10}$. Thus, in acidic region,

$$(g-1)+(g-2)a_H(Ka_2)^{-1}+(g-3)a_H^2(Ka_1Ka_2)^{-1}=0 \quad (11\text{-}3)$$

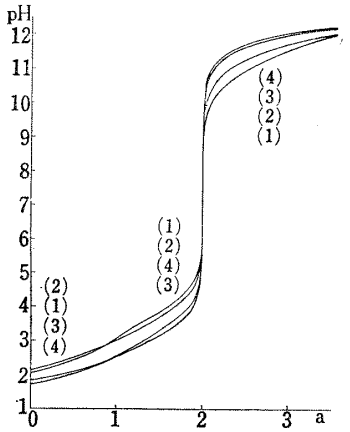
and, in alkaline region, the same equation as (11-2) holds.

It is easy to calculate each acid dissociation constant from one point (a_H, g) of the titration data, using (11-1) and (11-2). In order to calculate Ka_1 and Ka_2 from (11-3), two points in acidic region on the titration curve are connected with each other, and simultaneous equations are solved.

Treatment of UV spectral data will be explained in the following paragraph.

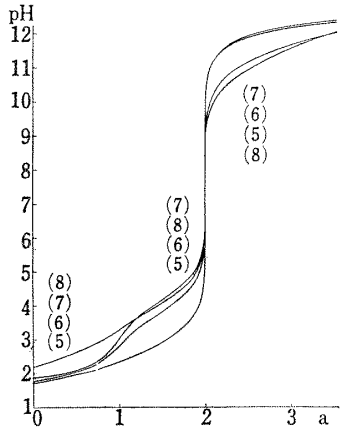
Results and Discussion

The titration curves of INH and related compounds are shown in Figs. 3a and 3b.



(1) INH+2HNO$_3$
(2) NH+2HNO$_3$
(3) PH+2HNO$_3$
(4) BH+2HNO$_3$
titrated with N NaOH

Fig. 3a. Titration Curves 1



(5) PNBH+2HNO$_3$
(6) INA+2HNO$_3$
(7) N-Me-INH+2HNO$_3$
(8) N′-Me-INH+2HNO$_3$
titrated with N NaOH

Fig. 3b. Titration Curves 2

Table II. The pKa Values of INH and Related Compounds

INH	$pKa_1 = 2.13 \pm 0.03$	(pH 2.05～3.32)[a]
	$pKa_2 = 3.81 \pm 0.01$	(pH 3.39～4.66)
	$pKa_3 = 11.03 \pm 0.02$	(pH 9.56～11.62)
NH	$pKa_1 = 2.26 \pm 0.04$	(pH 2.13～3.24)
	$pKa_2 = 3.63 \pm 0.02$	(pH 3.31～4.57)
	$pKa_3 = 11.49 \pm 0.01$	(pH 10.18～12.03)
PH	$pKa_1 = 1.26 \pm 0.07$	(pH 1.84～2.78)
	$pKa_2 = 3.07 \pm 0.01$	(pH 2.85～3.97)
	$pKa_3 = 12.25 \pm 0.02$	(pH 11.10～12.17)
BH	$pKa_2 = 3.27 \pm 0.01$	(pH 2.70～3.92)
	$pKa_3 = 12.53 \pm 0.04$	(pH 11.05～12.09)
PNBH	$pKa_2 = 2.90 \pm 0.01$	(pH 2.26～3.62)
	$pKa_3 = 11.28 \pm 0.04$	(pH 9.90～11.99)
INA	$pKa_2 = 3.82 \pm 0.01$	(pH 3.07～4.46)
	$pKa_3 > 15$	
N-Me-INH	$pKa_1 = 1.03 \pm 0.12$	(pH 1.83～3.52)
	$pKa_2 = 4.17 \pm 0.01$	(pH 3.63～4.98)
N′-Me-INH	$pKa_1 = 2.46 \pm 0.04$	(pH 2.21～3.56)
	$pKa_2 = 4.04 \pm 0.01$	(pH 3.65～4.89)
	$pKa_3 = 10.96 \pm 0.03$	(pH 9.57～11.10)

a) The pH ranges used in calculation are indicated in parentheses.

The calculated values of pKa are listed in Table II with the mean errors and the pH ranges used.

It is clear from the results of pH titration in acidic region (pH 1～6) that protons can be attached to a compound in the same number as the sum of pyridine ring and hydrazine. As to hydrazine, it is most probable that a proton is captured by the terminal amino nitrogen (2-N). pKa_2 of BH and PNBH, therefore, can be easily assigned to that of the hydrazine nitrogen, and pKa_2 of INA to that of the pyridine nitrogen. But, as INH, NH, PH, N-Me-INH and N′-Me-INH have two pKa's in this region, it is necessary to consider from the ultraviolet absorption spectra which is assigned to the pKa of pyridine nitrogen. The ultraviolet spectra of INH and related compounds in media of various pH are shown in Fig. 4.

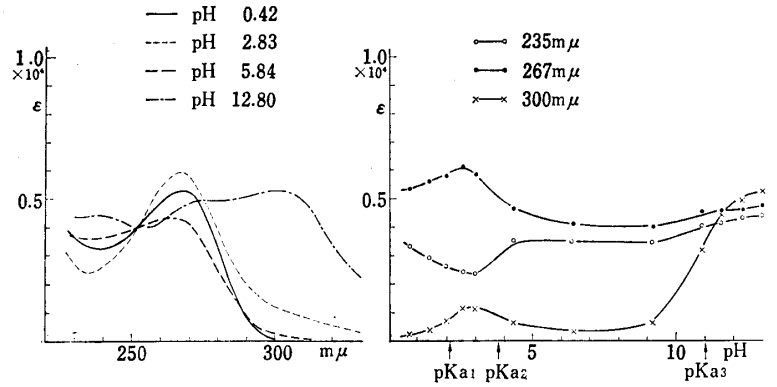
Jellinek, *et al.* investigated the ultraviolet spectra of nicotinamide[13] (NA), picolinamide[14] (PA) and isonicotinamide[14] (INA), and recognized that each absorbance of them increased when a proton was bound to the pyridine ring, similarly to pyridine itself[15] and its alkylderivatives.[15] Their results and the results for the corresponding hydrazides obtained by the authors are tabulated in Table III.

Table III. Selective Absorption of Pyridinecarbonic Acid Amides and the Corresponding Hydrazides

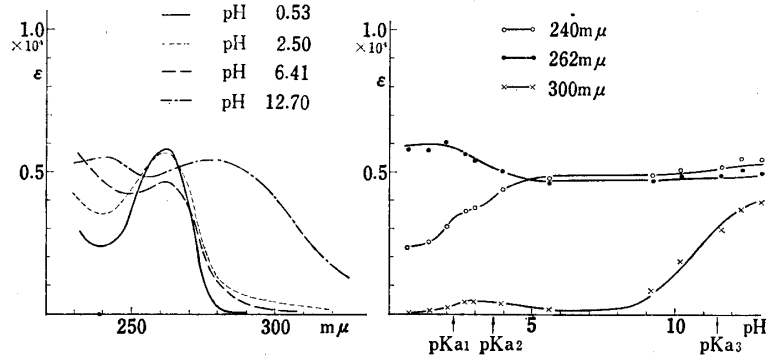
Compound	pKa_1	pKa_2	λ	$\varepsilon \times 10^{-3}$ at pH of			Reference
			mμ	pKa_1-1	pKa_2-2	neutral.	
INH	2.13[a]	3.81[c]	267	5.5	6.0	4.0	
INA	<−1.0[b]	3.61[c]	260	<4.8	4.8	2.2	14)
		3.82[c]	263		4.9	2.5	
NH	2.26[a]	3.63[c]	262	5.9	5.8	4.7	
NA	0.5[b]	3.35[c]	261.5	5.9	4.8	2.9	13)
PH	1.26[c]	3.07[a]	267	>7.0	5.7	6.0	
PA	<−1.0[b]	2.10[c]	265.5	<8.0	8.0	4.1	14)

a) pKa (H) *b*) pKa (A_1) *c*) pKa (P)

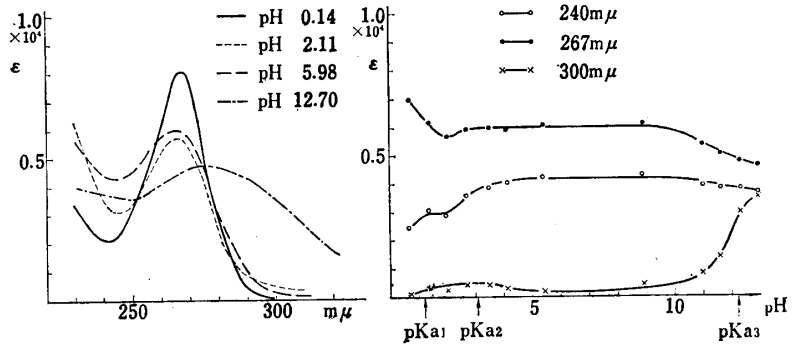
13) H.H.G. Jellinek, M.G. Wayne : J. Phys. Chem., **55**, 174 (1951).
14) H.H.G. Jellinek, J.R. Urwin : *Ibid.*, **58**, 548 (1954).
15) H.C. Brown, X.R. Mihm : J. Am. Chem. Soc., **77**, 1723 (1955).



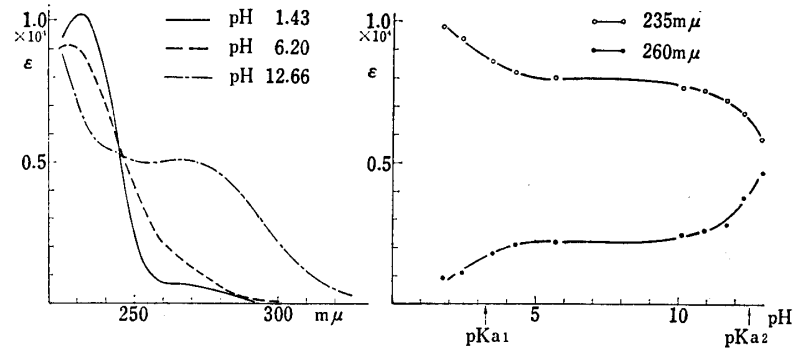
a isonicotinoylhydrazine (INH)



b nicotinoylhydrazine (NH)

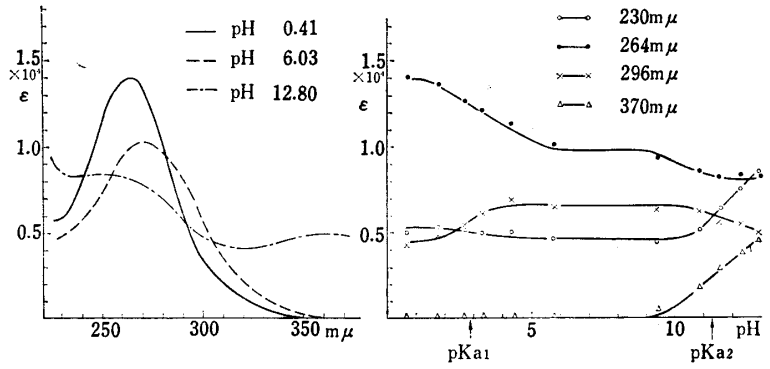


c picolinoylhydrazine (PH)

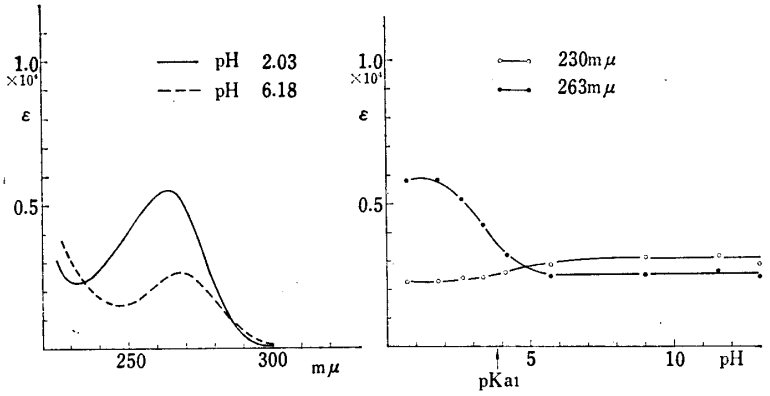


d benzoylhydrazine (BH)

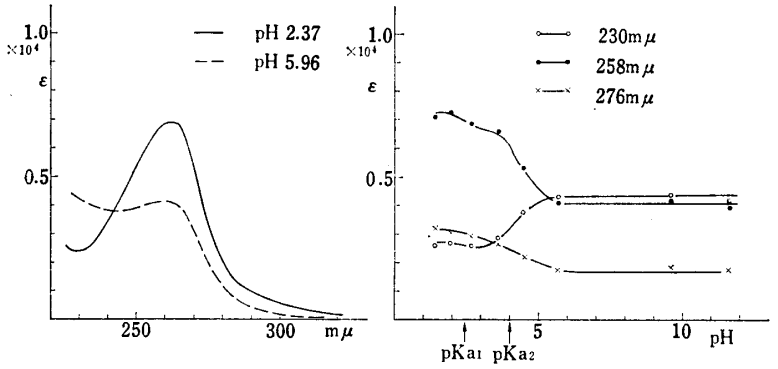
Fig. 4. (A) Characteristic Absorption Spectra and Their Changes *vs.* pH of INH and Related Compounds



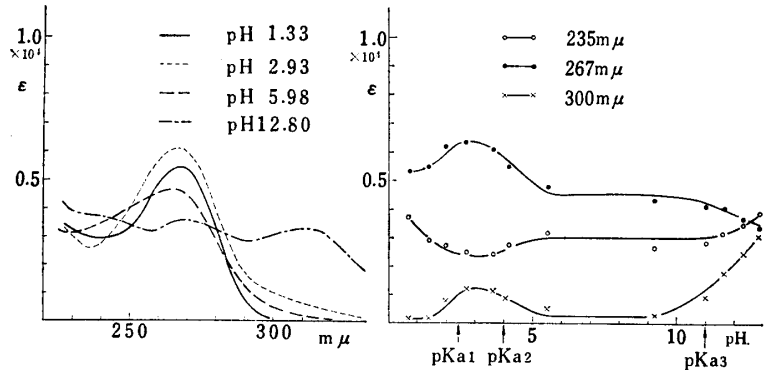
e *p*-nitrobenzoylhydrazine (PNBH)



f isonicotinamide (INA)



g 1-isonicotinoyl-1-methylhydrazine (N-Me-INH)



h 1-isonicotinoyl-2-methylhydrazine (N'-Me-INH)

Fig. 4. (B) Characteristic Absorption Spectra and Their Changes *vs.* pH of INH and Related Compounds

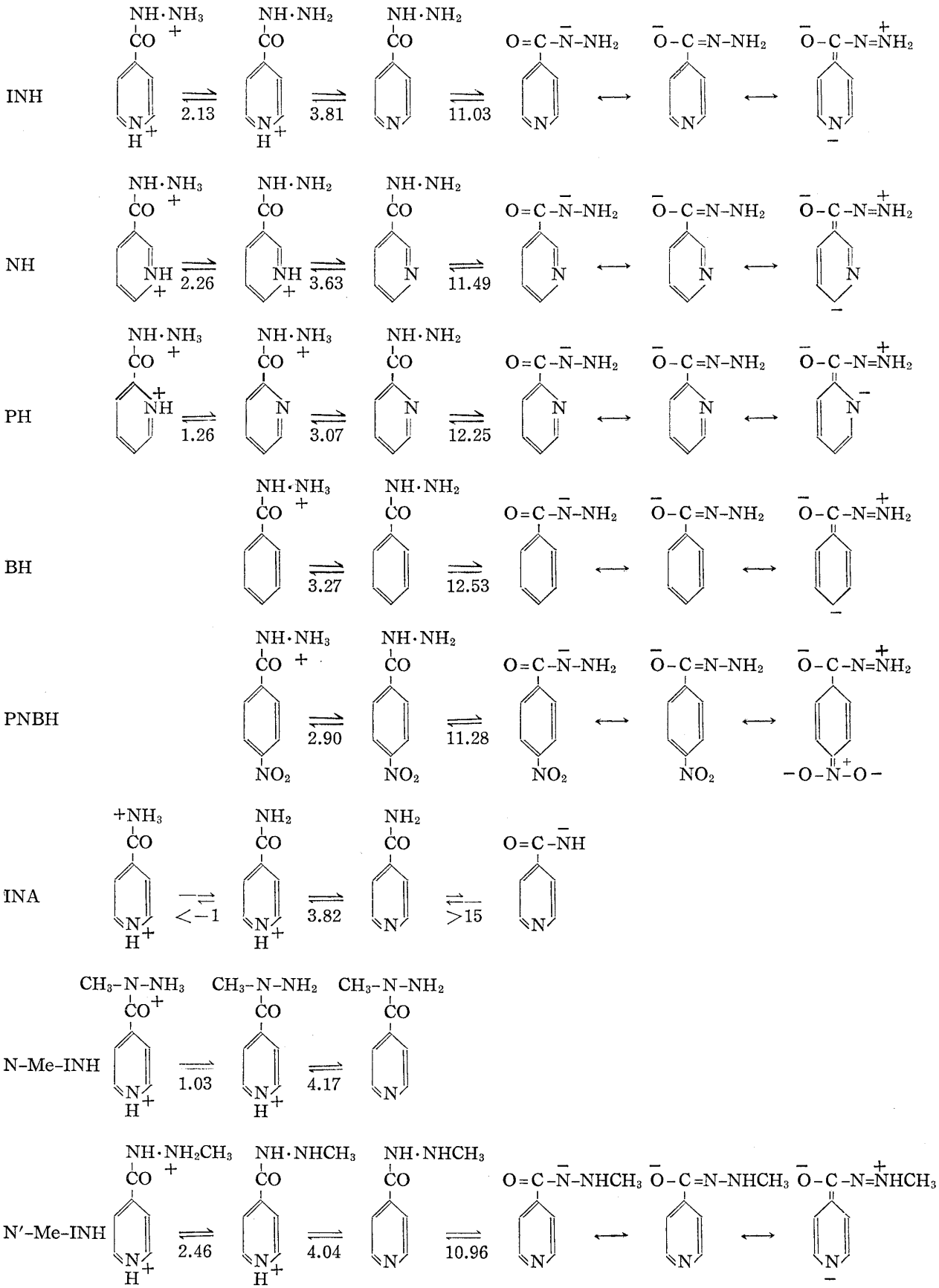
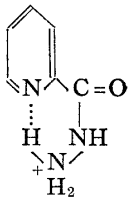


Chart 1.

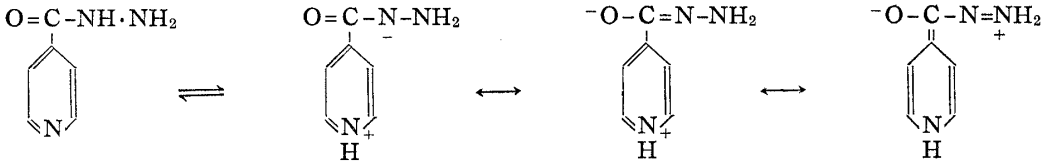
Here, pKa(H), pKa(P), and pKa(A) represent pKa of terminal nitrogen of hydrazide, of pyridine nitrogen, and of amide nitrogen respectively. pKa(A_1) corresponds to the association of a proton to amide nitrogen, while pKa(A_2) to the dissociation of a proton from the nitrogen.

Fig. 4a, 4b, 4c and Table III illustrate a good parallelism about values of λ_{max}, ε_{max} and pKa in acidic region between each amide and the corresponding hydrazide. In addition, the association of a proton to terminal amino nitrogen does not seem to exert such an important influence on the absorbance. As to INH and NH, consequently, it is most certain that pKa_2 is pKa(P) and pKa_1 is pKa(H). In the case of PH, on the other hand, as the absorbance increase sharply nearby the pH equal to pKa_1, it is probable that pKa_1 is pKa(P) and pKa_2 is pKa(H). It may be probably because of the formation of a hydrogen bonding that pKa(P) of PH is considerably smaller than that of PA.



Absorption maximum of all hydrazides except N-Me-INH are shifted to a longer wave length in alkaline region, and this phenomenon is surely due to the prolongation of a conjugated system caused from the dissociation of a proton from the nitrogen adjacent to a carbonyl group (1-N, A). Consequently, pKa_3 must be pKa(A_2). The results of the considerations mentioned above are summarized in Chart 1.

In addition, for the more precise treatment, a tautomerism in aqueous media might be considered.[16,17] For instance, a form HZ of INH will contain the following structures.



neutral form dipolar form

The authors express their deep gratitude to Professor Emeritus M. Ishidate for his continuous encouragement throughout this work. They are also indebted to Dr. K. Tsutsui, Toa Denpa Co., for his pertinent advice about pH measurement, to Prof. M. Ishihara, Kyoritsu Pharmaceutical College, for his assistance in UV measurement, to Mr. S. Mizutani, Tokyo Kasei Co., for his supply of chemical materials, and to Dr. E. Kimura and co-workers for elemental analyses.

Summary

Acid dissociation constants of isonicotinoylhydrazine (INH) and seven related compounds were determined by means of pH titration (ionic strength 1.0). Values of pKa obtained were as follows :

INH : 2.13, 3.81, 11.03. nicotinoylhydrazine (NH) : 2.26, 3.63, 11.49. picolinoylhydrazine (PH) : 1.26, 3.07, 12.25. benzoylhydrazine (BH) : 3.27, 12.53. *p*-nitrobenzoylhydrazine (PNBH) : 2.90, 11.28. isonicotinamide (INA) : 3.82. 1-isonicotinoyl-1-methylhydrazine (N-Me-INH) : 1.03, 4.17. 1-isonicotinoyl-2-methylhydrazine (N'-Me-INH) : 2.46, 4.04, 10.96.

The ultraviolet spectra of these compounds at various pH were measured, and the results showed a good parallelism with the pKa values. Some informations about their structures in aqueous solutions were obtained from the spectral data.

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