Studies on Nicotinoids as an Insecticide

Part VII. Cholinesterase Inhibition by Nicotinoids and Pyridylalkylamines—Its Significance to Mode of Action*

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The inhibition of house fly head cholinesterase by twelve nicotinoids and twenty six pyridylalkylamines was investigated in relation to their ionizations and toxicities to house flies, *Musca domestica* L. The significant correlation between toxicity and inhibition, and the competitive nature of inhibition indicate that there are some similarities between the receptor for toxic action and the active center of cholinesterase for combining the molecule. Cholinesterase inhibition is shown to be caused by the anionic site binding on the enzyme of the cationic head of the molecule. Hence, the toxic action of nicotine and 3-pyridyl-methylamines is best explained by postulating the nerve receptor carrying the anionic site, which is partly similar to the anionic site of the active center in cholinesterase. The effect on fly nerve activity can also be correlated with the chemical structure. It is shown that the highly toxic nicotinoids should be provided with high but not too high basicity. Dihydronicotyrine having pK_a' near the pH of insect body fluid shows the highest toxicity. This can be understood in terms of the preference of free base for penetration and the requirement of ionization for the interaction with the receptor.

For mammals nicotine is an autonomic blocking agent and acts like acetylcholine, but only at ganglia and neuromuscular junction, initially stimulating and then depressing them. Those symptoms caused by applied acetylcholine which resemble those in nicotine poisoning are called "nicotinic." The central nervous system of the insect, which is susceptible to organophosphates, carbamates, and nicotine poisoning probably uses acetylcholine as a transmitter substance at synapse. Nicotine is ionized nearly 90% at physiological pH and the ionized form has been assumed *a priori* to be an active form for mammals by analogy from quaternary nitrogen of acetyl-

choline. On the other hand, the study of structure-insecticidal activity relationship of nicotinoids for insects by Yamamoto *et al.*¹⁾ revealed that they should be fairly strong bases but not quaternarized and they should mimic acetylcholine with respect to their molecular dimension and electronic make-up. At a glance it seemed to be strange that more ionizable nicotinoids were more insecticidal because there are considerable evidences that the ion penetrates insect ganglia and nerve very slowly, and it can be expected that the ionic toxicants have much less effect upon the insect nervous system than their non-ionic analogs. We can see an example in Amiton

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1) I. Yamamoto, H. Kamimura, R. Yamamoto, S. Sakai and M. Goda, *Agr. Biol. Chem.*, **26**, 709 (1962).

and Tetram.²⁾ In such cases, the ionizable moiety is an auxiliary part in the toxic molecule. In the case of nicotinoids, when we assume that the ionizable moiety is an essential part in the molecule and that the ionized form interacts with the receptor, the seeming anomaly can be solved.

A direct approach to verify the interaction between toxicant and the receptor is needed. An attempt to fractionate insect nerve components was tried by Matsumura and Hayashi.³⁾ However, because of the difficulty of isolating the receptor site from the nerve, we used house fly head cholinesterase as a model of the receptor. It has been understood that acetylcholine interacts with both cholinesterase and the nerve receptor and nicotine with the same receptor. Therefore, the interaction of nicotine and other nicotinoids with the enzyme is expected to reflect, at least partly, the interaction with the receptor. We have shown⁴⁾ that cationic head of nicotine ion interacts with the anionic site in the active center of cholinesterase. In the present paper the inhibition of the house fly head cholinesterase by twelve nicotinoids and twenty six pyridylalkylamines was investigated in relation to their ionizations and toxicities to house flies, *Musca domestica* L.

MATERIALS AND METHODS

Chemicals. The compounds used are listed in Table I. Preparation of *dl*-nicotine will be reported in the following paper.⁵⁾ *dl*-Cotinine (5) was prepared according to the method of Sugawara *et al.*⁶⁾ by catalytically reducing ethyl β -nicotinoylpropionate with methylamine in alcoholic solution over Raney nickel (W-5) at 150°C (initial pressure 65 atm). We obtained crystalline cotinine by the following procedure.

The reduction product, showing four spots on thin layer chromatography using a solvent system of *n*-hexane and acetone (7:3) and silica gel (two majors, 0.65 and 0.32 and two minors, 0.79 and 0.42 in *R_f* value), was column chromatographed on silica gel by using a solvent system of *n*-hexane with increasing ratio of acetone (0~100%). The fraction containing the desired product (*R_f* 0.32) gave crystalline cotinine of mp 52°C from petroleum ether-benzene. Yield, 55% *pK_a'* 4.37. $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu(\epsilon)$ 261.6 (9560). Picrate, mp 122°C from methanol-acetone. *Anal.* Found: C, 46.96; H, 3.92; N, 16.98. Calcd. for $\text{C}_{10}\text{H}_{12}\text{ON}_2 \cdot \text{C}_6\text{H}_3\text{O}_7\text{N}_3$: C, 47.41; H, 3.73; N, 17.28%.

Myosmine (6) was prepared according to the method of Korte *et al.*⁷⁾ from *N*-nicotinoyl-2-pyrrolidone and ethyl nicotinate. Crystalline myosmine of mp 44°C and bp 138°C (15 mmHg) [44°C (7 mmHg) by Korte].⁷⁾ *pK_{a1}'* 5.47 and *pK_{a2}'* 2.85. $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu(\epsilon)$ 234 (8050). Picrate, mp 188°C from acetone (184°C¹¹⁾). Anabasine (7) was prepared by the same method as myosmine except using *N*-nicotinoyl-2-piperidone instead of *N*-nicotinoyl-2-pyrrolidone. Bp 129°C (6 mmHg) [106~107°C (2 mmHg)⁹⁾]. *pK_{a1}'* 6.65 and *pK_{a2}'* 2.80. Picrate, mp 167°C from acetone (167°C⁸⁾). *N*-Methylanabasine (10) was obtained by methylating anabasine (11) with formaldehyde and formic acid according to conventional procedure. Bp 108~110°C (11 mmHg). $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu(\epsilon)$ 261 (2760). *pK_{a1}'* 7.90 and *pK_{a2}'* 2.94. Picrate, mp 205~206°C. *Anal.* Found: C, 43.95; H, 3.68; N, 17.47. Calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_2 \cdot 2\text{C}_6\text{H}_3\text{O}_7\text{N}_3$: C, 43.54; H, 3.50; N, 17.66%. The preparations of other nicotinoids¹⁾ and pyridylalkylamines⁹⁾ have been reported in the previous papers. *pK_a'* at 25°C and *R_f* values are listed in Table II. *pK_a'* at 37°C are shown in Table III.

Insecticidal activity. The material in acetone solution was topically applied to house flies, *Musca domestica* L. and the mortalities were recorded after 24 hr. The detailed procedure has been described in the foregoing paper.⁹⁾ Insecticidal activities of nicotinoids are summarized in Table II with the regression equations.

Anticholinesterase activity. The enzyme preparation from house fly heads and anticholinesterase

2) R. D. O'Brien, *J. Econ. Entomol.*, **52**, 812 (1959).

3) F. Matsumura and M. Hayashi, "Experimental approaches to pesticide metabolism, degradation and mode of action," U.S.-Japan Seminar, August 1967, p. 231.

4) Y. Soeda and I. Yamamoto, *Agr. Biol. Chem.*, **32**, 568 (1968).

5) Y. Soeda and I. Yamamoto, in preparation.

6) S. Sugawara, T. Tatsuno and T. Kamiya, *Pharm. Bull. Japan*, **2**, 39 (1954).

7) F. Korte und H.-J. Schulze-Steinen, *Chem. Ber.*, **95**, 2444 (1962).

8) H. Kamimura and I. Yamamoto, *Agr. Biol. Chem.*, **27**, 450 (1963).

9) Y. Soeda and I. Yamamoto, *ibid.*, **32**, 747 (1968).

TABLE I. CHEMICAL STRUCTURES OF COMPOUNDS USED.*

Nicotinoids			Pyridylalkylamines
<i>s</i> -	(1)	$\text{Py}-\text{CH}_2-\text{NH}_2$	(15)	$\text{Py}-\text{CH}_2-\text{N}$	(28)
<i>dl</i> -	(2)	$\text{Py}-\text{CH}_2-\text{NHCH}_3$	(16)	$\text{Py}-\text{CH}_2-\text{N}$	(29)
(3)	$\text{Py}-\text{CH}_2-\text{NHC}_2\text{H}_5$	(17)	$\text{Py}-\text{CH}_2-\text{N}$	(30)
<i>dl</i> -	(4)	$\text{Py}-\text{CH}_2-\text{NH}(n\text{-C}_3\text{H}_7)$	(18)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{NH}_2$	(31)
<i>dl</i> -	(5)	$\text{Py}-\text{CH}_2-\text{NH}(i\text{-C}_3\text{H}_7)$	(19)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{NHC}_2\text{H}_5$	(32)
(6)	$\text{Py}-\text{CH}_2-\text{NH}(n\text{-C}_4\text{H}_9)$	(20)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_3)_2$	(33)
(7)	$\text{Py}-\text{CH}_2-\text{N}(\text{CH}_3)_2$	(21)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$	(34)
<i>dl</i> -	(8)		(22)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}$	(35)
<i>dl</i> -	(9)	$\text{Py}-\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$	(23)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}$	(36)
<i>dl</i> -	(10)		(24)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{NHCH}_3$	(37)
<i>s</i> -	(11)	$\text{Py}-\text{CH}_2-\text{N}(n\text{-C}_3\text{H}_7)_2$	(25)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_3)_2$	(38)
<i>dl</i> -	(12)	$\text{Py}-\text{CH}_2-\text{N}(i\text{-C}_3\text{H}_7)_2$	(26)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{NHCH}_3$	(39)
<i>s</i> -	(13)	$\text{Py}-\text{CH}_2-\text{N}(n\text{-C}_4\text{H}_9)_2$	(27)	$\text{Py}-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_3)_2$	(40)
	<i>s</i> -	(14)				

* Py is an abbreviation for 3-pyridyl group. Trivial names of nicotinoids are as follows; Nicotine (1), nicotyrine (3), dihydronicotyrine (4), cotinine (5), myosmine (6), anabaseine (7), nornicotine (8), anabasine (9), N-methylanabasine (10), nicotine monomethiodide (11), nicotine isomethiodide (13), nicotine dimethiodide (14). *s*- denotes the compounds derived from *s*- or

TABLE II. TOXICITY, BASICITY AND *Rf* VALUE OF NICOTINOIDS

Compound	Toxicity*			Basicity**		<i>Rf</i> *** value
	Regression equation	LD ₅₀ (μmole/female)	Relative toxicity	p <i>K</i> _{a1} '	p <i>K</i> _{a2} '	
(1)	Y=5+4.81(X-1.114)	0.080	1.00	7.90	3.10	0.38
(2)	Y=5+5.28(X-1.097)	0.077	1.04	7.90	3.10	0.38
(3)	Y=5+3.29(X-1.845)	0.44	0.18	4.75	2.40	0.69
(4)	Y=5+4.15(X-1.041)	0.069	1.16	7.45	2.90	0.43
(5)	Y=5+2.63(X-2.748)	3.18	0.025		4.37	0.18
(6)	Y=5+3.82(X-1.532)	0.22	0.36	5.47	2.85	0.39
(7)	Y=5+8.80(X-1.204)	0.10	0.80	6.65	2.80	0.41
(8)	Y=5+4.32(X-1.653)	0.30	0.27	9.00	3.35	0.09
(9)	Y=5+6.08(X-1.346)	0.14	0.57	8.72	3.10	0.14
(10)	Y=5+7.05(X-1.146)	0.080	1.00	7.90	2.94	0.36

* Toxicity to house fly, *Musca domestica* L., by the topical application method. X=dosage (μg) in logarithm. Y=mortality in probit.

** p*K*_a' obtained by a titration method¹⁾ at 25°C.

*** *Rf* value for the thin layer chromatography on Merk's TLC-plate Gel F₂₅₄ using a solvent system consisting of *n*-hexane : acetone : triethylamine (10 : 10 : 1 v/v).

assay using acetylcholine as substrate have been described in the previous paper.⁴⁾ For comparison of many compounds, the concentration of inhibitor for 50% inhibition of the enzyme activity, *I*₅₀, was used. *I*₅₀ is calculated by probit method using data from two repetitions for 4 to 5 different concentrations of an inhibitor. The substrate levels (1.0 and 2.0 mM) were used. The results are summarized in Table III. In the following discussion, we postulate that the compounds used might inhibit the enzyme competitively. Competitive nature of the inhibition for nicotine and its methiodide has been studied by kinetic study.⁴⁾ In this paper, instead of applying such study for compound by compound, we take the ratio of *I*₅₀ at two different concentrations of the substrate (1.0 and 2.0 mM), which is called competitive index (CI) in Table III. It should be 2.0 for competitive inhibition. Most of the compounds locate in the range between 1.5 and 2.5 and the compounds deviated from the range are low toxic compounds except few. Therefore, the following discussion will not be seriously affected by this results.

RESULTS AND DISCUSSION

In a previous paper⁴⁾ it was shown that the cationic head of nicotinium ion interacts with the anionic site in the active center of cholinesterase by a kinetic study and the pH dependency of the enzyme inhibition. The structure-insecticidal activity relationship of

FIG. 1. Relation between Relative Toxicity and Anticholinesterase Activity.

A dash line indicates correlation for nicotine. Relative toxicity is the ratio of LD₅₀ of *l*-nicotine to that of a compound. p*I*₅₀ at 37°C, pH 7.4 and 2.0 mM of acetylcholine.

- Nicotinoids
- 3-Pyridylmethylamines
- △ 3-Pyridylethylamines
- 3-Pyridylbutylamines
- ▲ Compounds (22) and (24)

pyridylalkylamines has been reported.⁹⁾ Assuming that nicotinoids and pyridylalkyl-

TABLE III. ANTICHOLINESTERASE ACTIVITY, TOXICITY AND BASICITY OF NICOTINOIDS AND PYRIDYLALKYLAMINES

Anticholinesterase activity*							
Compound	Ach 1.0 mM		Ach 2.0 mM		CI	Relative toxicity**	Basicity*** (p <i>K</i> _{A1'} at 37°C)
	I ₅₀ (mM)	pI ₅₀	I ₅₀ (mM)	pI ₅₀			
Nicotinoids							
(1)	1.4	2.85	2.6	2.58	1.9	1.00	7.66
(2)			0.62	3.21		1.04	7.66
(3)	1.5	1.83	2.3	1.64	1.5	0.18	4.51
(4)	0.23	3.64	0.35	3.46	1.6	1.16	7.21
(5)	200.	0.70	260.	0.58	1.3	0.025	
(6)	11.	1.96	6.8	2.17	0.6	0.36	5.23
(7)	2.6	2.58	3.5	2.46	1.4	0.80	6.41
(8)	9.6	2.02	9.7	2.01	1.0	0.27	8.76
(9)	4.4	2.36	3.9	2.41	0.9	0.57	8.48
(10)	0.43	3.37	0.72	3.14	1.7	1.00	7.66
(11)	0.50	3.30	1.0	3.00	2.0		
(12)			0.64	3.19			
(13)	0.92	3.04	1.3	2.89	1.4		5.71
(14)	10.(30%)		10.(30%)				
Pyridylalkylamines							
(15)	110.	0.95	100.	1.0	1.0	0.029	8.00
(16)	17.	1.77	31.	1.51	1.8	0.30	8.30
(17)	5.7	2.24	7.1	2.15	1.3	0.34	8.96
(18)	11.	1.96	17.	1.77	1.5	0.15	8.38
(19)	2.1	2.68	3.9	2.41	1.9	0.46	8.46
(20)	11.	1.96	23.	1.64	2.0	0.16	8.66
(21)	1.8	2.75	5.1	2.29	2.8	0.46	7.76
(22)	1.1	2.96	3.0	2.52	2.7	0.085	8.42
(23)	0.45	3.35	0.89	3.05	2.0	0.45	8.12
(24)	3.0	2.52	7.7	2.11	2.6	0.10	7.96
(25)	1.2	2.92	1.7	2.77	1.4	0.16	8.13
(26)	0.13	3.98	0.31	3.51	2.4	0.44	8.66
(27)	1.4	2.85	2.8	2.55	2.0	0.28	8.08
(28)	0.80	3.10	1.4	2.85	1.8	0.43	8.11
(29)	1.0	3.00	1.5	2.83	1.5	0.79	8.16
(30)	120.(21%)		64.(23%)			0.038	5.76
(31)	100.	1.0	150.	0.82	1.5	0.065	8.99
(32)	8.8	2.05	11.	1.96	1.3	0.098	8.92
(33)	5.9	2.23	11.	1.96	1.9	0.42	8.28
(34)	1.6	2.80	4.0	2.40	2.5	0.13	8.54
(35)	5.9	2.23	12.	1.92	2.0	0.22	8.90
(36)	2.7	2.57	8.4	2.08	3.1	0.14	8.57
(37)	2.5	2.60	11.	1.95	4.4	0.13	9.96
(38)	2.3	2.64	8.6	2.07	3.7	0.31	8.76
(39)	2.8	2.55	9.6	2.02	3.3	0.071	9.68
(40)	2.1	2.68	7.6	2.12	3.6	0.23	9.03

* Anticholinesterase assay was done at two substrate levels (1.0 mM and 2.0 mM of acetylcholine, Ach) at pH 7.4 and 37°C. I₅₀ is shown by the concentration in the enzyme reaction mixture. pI₅₀ indicates -log I₅₀. CI is the ratio of I₅₀ at 2.0 mM Ach to I₅₀ at 1.0 mM Ach.

** Based on LD₅₀ to house fly, *Musca domestica* L., by topical application method. Corrected on molar basis.

*** Basicity at 37°C was calculated by the method of Albert (*Pharmacol. Rev.*, **4**, 136 (1952)), from the data measured at 25°C.

amines behave similarly to nicotine on the enzyme, we compared their I_{50} in relation to their toxicity and ionization. Figure 1 demonstrates the correlation between relative toxicity (nicotine is taken as 1.0) and anticholinesterase activity (pI_{50}). For nicotinoid series including dihydronicotyrine (4), nicotine (1, 2), N-methylanabasine (10), anabaseine (7), anabasine (9), myosmine (6), nornicotine (8), nicotyrine (3), and cotinine (5), it is seen that the higher the inhibition, the higher the toxicity becomes. 3-Pyridylmethyamines constitute another group. Though there are some deviations, the compounds having toxicity higher than a given level (say, relative toxicity 0.4) give higher inhibition of the enzyme. This is seen for piperidyl (29), pyrrolidyl (28), dimethylamino (21), diethylamino (23), di-*i*-propylamino (26), and *i*-propylamino (19) compounds. On the other hand, low inhibitory 3-pyridylmethyamines, that is, ethylamino (17), *n*-propylamino (18), *n*-butylamino (20), amino (15), and morpholino (30), which are of either primary, secondary or low basic amine, give lowered toxicity.

The 3-pyridylmethyamine moiety, proposed as the essential moiety for toxic action, seems unessential for the enzyme inhibition, but still preferable. 3-Pyridylethylamines and 3-pyridylbutylamines (not provided with the essential moiety) are usually found to be less toxic than expected from the inhibitory power as shown in Fig. 1. Figure 1 or Table III shows that 3-pyridylmethyamines are more inhibitory and more insecticidal than the corresponding 3-pyridylethylamines having the same substituents on the nitrogen (17 vs. 32; 21 vs. 33; 23 vs. 34; 28 vs. 35; 29 vs. 36). Furthermore, when 2-, 3-, and 4-pyridylmethyl-N,N-diethylamines are compared for their toxicity and inhibitory power, 3-pyridyl compound, which is provided with the essential moiety, gives the highest inhibition as well as toxicity (Fig. 2).

From the above data there seems to be an apparent correlation among the chemical

FIG. 2. Relation among Structure, Toxicity and Fly Head Cholinesterase Inhibition of Pyridylmethyl-N, N-diethylamines.

Enzyme inhibition —●—
Relative toxicity —○—
 pI_{50} at 37°C, pH 7.4 and 2.0 mM acetylcholine.

structure, the toxicity, and the enzyme inhibitory power. However, this does not mean that mechanism of toxic action is the cholinesterase inhibition, because the concentration required to kill the house flies is far lower than the concentration required for inhibition. Rather the change of toxicity is the result of some changes in the chemical and physical properties of the molecule, and the latter changes are again reflected on the enzyme inhibitory power. So we can say that there are some similarities between the receptor for toxic action and the active center of cholinesterase.

The mechanism of enzyme inhibition has been discussed with nicotine and nicotine monomethiodide.⁵¹ In addition, the pH dependency of the enzyme inhibition by 3-pyridylmethyl-N,N-diethylamine (23) is shown in Fig. 3, indicating that the higher the pH (the lower the ionization), the lower the inhibition becomes. Furthermore, the relation among basicity, ionization, and anticholin-

FIG. 3. pH Dependency of Fly Head Cholinesterase Inhibition by 3-Pyridylmethyl-N,N-diethylamine (—○—) and its ionization (---).

2.0 mM acetylcholine and 0.2 mM of the inhibitor at 37°C.

FIG. 4. Relation among Basicity (pK_a'), Ionization, and Fly Head Cholinesterase Inhibition (pI_{50}).

pI_{50} at 37°C, pH 7.4, and 2.0 mM acetylcholine.

- Nicotinoids
- 3-Pyridylmethylenamines
- △ 3-Pyridylethylamines
- Pyridylbutylamines
- ▲ Compounds (22) and (24)

esterase activity is shown in Fig. 4. For tertiary amine type of compounds, that is nicotinoids (A), N,N-disubstituted 3-pyridylmethylenamines (B), and 3-pyridylethylamines (C), general tendency is that the higher the pK_a' (the higher the ionization), the higher the pI_{50} (inhibition) becomes. However, pri-

mary (15, 31) and secondary amines (8, 9, 16, 17, 18, 19, 20, 32, 37, 39) show lowered inhibition, though they are of high basicity and highly ionized. This may be the same case as studied by Wilson¹⁰⁾ who showed that in ethanolamine series, the anticholinesterase activity becomes low, when the numbers of methyl group on nitrogen decrease. Thus, the anionic site binding on the enzyme seems to require some steric factor and/or hydrophobic interaction which reinforce the Coulombic interaction. The anomalous positions for nornicotine (8) and anabasine (9) in Fig. 4 can be explained from their secondary amine nature.

From the above results and discussion, the toxic action of nicotinoids and 3-pyridylmethylenamines is best explained by postulating the receptor carrying the anionic site which is at least partly similar, to the anionic site of the active center in cholinesterase.

Expecting to remove factors of metabolism and excretion, we have made a preliminary attempt¹¹⁾ to obtain the effect on house fly nerve activity of a few nicotinoids and 3-pyridylmethylenamines. Details about experimental procedure were described by Narahashi.¹²⁾ In brief, the effect on fly nerves was evaluated by counting the action potential outbreak frequencies with a spike counter, when the test compounds were applied. The percentage of house flies giving frequencies higher than a given standard was taken as the response. As shown in Fig. 5, the highly toxic nicotinoids, that is nicotine (1), dihydronicotyrine (4), as well as 3-pyridylmethylenamines, that is dimethylamino (21), diethylamino (23), pyrrolidyl (28), and piperidyl (29) give higher response, whereas low basic (30) or primary amine (15) type (hence low toxic) gave far lower response.

From surveying the insecticidal activities

10) I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).

11) The data of this part; I. Yamamoto and K. Ishida, not published (1965).

12) T. Narahashi, *J. Med. Sci. Biol.*, **17**, 46 (1964).

FIG. 5. Dose—Response Relation of Nicotinoids and 3-Pyridylmethylamines.

Response: percentage of house flies giving the action potential outbreak frequencies by toxicant higher than a given standard.¹²⁾

	compound	relative toxicity
—●—	21	0.46
—▲—	4	1.16
—△—	23	0.45
—□—	29	0.79
—○—	1	1.0
—■—	28	0.43
—⊙—	30	0.038
—+—	15	0.029

on various kinds of insects, Yamamoto *et al.*^{1,13)} have deduced a general principle that all nicotinoids of high toxicity are highly basic. This is also true for house flies. Moreover, we have found that the toxicity became low when the basicity of a nicotinoid became too high (high ionization) as shown in Fig. 6. The extreme is nicotine monomethiodide (11) which is 100% ionized and almost non-toxic. The most toxic nicotinoid is dihydronicotyrine (4) having pK_a' 7.45, which is very close to the pH of the fly body fluid. If we assume the pH as 7.45, dihydronicotyrine is 50%

FIG. 6. Relation between pK_a' and Relative Toxicity of Nicotinoids.

ionized, while nicotine is 74% ionized. This bell shaped relation between toxicity and basicity (ionization) can be understood in terms of penetration and toxic interaction at the receptor. As discussed before, the free base form is more penetrable into the insect body (through the cuticle) and the target area (through the ion impermeable barrier), whereas the ionized form is responsible for toxic action. Therefore, we might roughly take (ionization %) $\times$ (free base %) as an index of gross relative toxicity, which gives a bell shape against pK_a' , qualitatively agreeing with the actual toxicities. This suggests that the balancing of polarity with lipophilic nature is required for good gross toxicity. A more detailed study from a quantitative point of view, using substituent constants relating electronic and hydrophobic nature, will be reported later. Dihydronicotyrine (4) seems to approach the maximum toxicity idealized from the above mentioned theory. Indeed dihydronicotyrine has been the most toxic among nicotinoids not only for house flies but also for other insects including aphids.

13) I. Yamamoto, *Advances in Pest Control Research*, **6**, 231 (1965).