原　著

Studies on Nicotinoids as an Insecticide. Part VIII. Physiological Activities of the Optical Isomers of Nicotinoids. Yoshinori Soeda* and Izuru Yamamoto (Laboratory of Pesticide Chemistry, Department of Agricultural Chemistry, Tokyo University of Agriculture, Setagaya, Tokyo). Received March 24, 1969. *Botyu-Kagaku* 34, 57, 1969.

6. ニコチノイド殺虫剤の研究（第8報）. ニコチノイド光学異性体の生理活性の比較　添田吉則*)・山本　出（東京農業大学農学部農芸化学科，東京都世田谷区）44. 3. 24 受理.

ニコチノイド光学異性体の昆虫への毒性を比較したところ，イエバエ，ワモンゴキブリおよびコクゾウに対し，ニコチンの両光学異性体の活性は等しく，モモコフキアブラムシおよびニカメイチュウに対しては，*l*-ニコチンは *d*-体の約2倍の活性を示した．イエバエ頭部アセチルコリンエステラーゼの阻害については，*d*-ニコチンは *l*-体の11.3倍，また *l*-ノルニコチンは *dl*-体の3.6倍の活性を示した．本結果および過去の実験事実を吟味し，両光学異性体は作用点において等しい活性を有するが，作用点到達への過程における差が生理活性の相異をもたらすものと推定した．

Introduction

Natural nicotine is levorotatory 1'-methyl-2'-(3-pyridyl)-pyrrolidine. *l*-Nicotine may be called L- according to the amino acid system or S- according to the system of Cahn, Ingold, and Prelog[1], the antipode being D- or R-. *l*-Nicotine is oxidized by potassium permanganate or silver oxide[2] to *l*-nornicotine, therefore, both have the same configuration. However, though *Nicotiana* plants always yield the optically pure *l*-nicotine ($[\alpha]_D^{20}=-169°$), the rotatory power of natural nornicotine is quite variable, the *l*-form being usually predominant($-17.7°$, $-39.7°$, $-45°$, $-70°$ and so on)[3], and the *d*-form being predominant in one case[4], Japanese *Nicotiana tabacum* L. ($[\alpha]_D^{24.6}=+20.16°$). Optically pure *l*-nornicotine has $[\alpha]_D^{23}=-88.8°$[5]. *d*-Nicotine was prepared by methylation of *d*-nornicotine obtained from excess *d*-nornicotine or by repeated fractional crystallizations of *dl*-nicotine *l*-tartarate[6]. Recently, microbial resolution of *dl*-nicotine gave 60～80% yield of *d*-nicotine[7].

Concerning the biochemical interaction of the optical isomers of nicotine, it was demonstrated by Kisaki and Tamaki[8] that the demethylation of nicotine was stereospecific and *d*-nicotine-$^{14}CH_3$ was diminished more rapidly than *l*-nicotine-$^{14}CH_3$ in the excised leaves of *Nicotiana tabacum*. The pharmacological actions, which were summarized in Table 3, of *d*- and *l*-nicotine have been studied by many investigators as discussed later, and *l*-nicotine has been described as being more toxic than the *d*-form in many textbooks. However on the toxicity to the insects, there have been only reports with the aphid, gammarus, drosophila and house fly.

In the present paper, in relation to the mode of insecticidal action of nicotine, the physiological activities of the optical isomers of nicotine against *Musca domestica* L. (house fly), *Periplaneta americana* L. (American cockroach), *Sitophilus zeamais* M. (rice weevil), *Hyalopterus arundinis* F. (mealy plum aphid), and *Chilo suppressalis* W. (rice stem borer), and the anticholinesterase activity were compared to examine whether any fundamental difference exist or not.

Materials and Methods

Chemical section.

l-Nicotine. Commercially available nicotine sulfate (Yashima Kagaku Co., Ltd.) was alkalinized and free nicotine was extracted with benzene. After drying with anhydrous sodium sulfate and evaporating, the residue was distilled to give optically pure *l*-nicotine boiling at 97～98°C/9 mmHg. $[\alpha]_D^{24}=-169.0°$ (C, 100).

dl-Nicotine. *l*-Nicotine (100g) was autoclaved at 200°C for 4 days in a aqueous solution (1*l*)

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adjusted to pH 4.0 with conc. sulfuric acid by the Kisaki's method[9]. The reaction mixture alkalized with sodium hydroxide was steam distilled to give the distillate (4*l*). After acidification of the distillate with hydrochloric acid, the condensed syrup was made alkaline and extracted with benzene. The residue of the benzene evaporation was distilled to give crude *dl*-nicotine (31.4g). 70% Perchloric acid (45g) was added to crude *dl*-nicotine to form the white precipitate, which was recrystallized twice from methanol (m.p. 211°C). After setting free, extraction and distillation, *dl*-nicotine (9.6g) was obtained as an almost optically pure one boiling at 119~120°C/18mmHg. $[\alpha]_D^{24} = -0.51°$ (C, 100).

d-Nicotine. 100% Optically pure *d*-nicotine, which was resolved by the microbial method[7], was kindly supplied by Dr. Kisaki. *d*-Dominant nicotine was also obtained by the chemical resolution mentioned below. By the Pictet's method[6], *dl*-nicotine (27.3g) was added dropwise under cooling to *d*-tartaric acid (50.5g) dissolved in water (40m*l*). The mixture was allowed to stand for 2 days in the refrigerator to give white precipitate. The resulting precipitate (24.1g) (m.p. 86~87°C), *l*-nicotine *d*-tartarate, was filtered off and the filtrate was set free to give *d*-dominant nicotine (18.0g) having $[\alpha]_D^{24} = +47.35°$ (C, 100). From *d*-dominant nicotine (5.0g) and *l*-tartaric acid (9.4g) in water (30m*l*), *d*-nicotine *l*-tartarate (m.p. 86~87°C) was obtained. After two more recrystallizations from water and set freeing, 91.4% optically pure *d*-nicotine (1.8g) was obtained. B.p. 100~101°C/10mmHg. $[\alpha]_D^{24} = +139.0°$ (C, 100.)

Nornicotine. *l*-Nornicotine, naturally occurred, was supplied by Dr. Kisaki. *dl*-Nornicotine was obtained from myosmine by reduction with sodium borohydride as described in the previous paper[10].

Nicotine monomethiodide. *l*-Nicotine monomethiodide was prepared by the same procedure as described in the previous paper[11]. M.p., 133~134°C. UV. λ_{max}^{MeOH}, 226 and 260 mμ. $[\alpha]_D^{24} = +27.22°$ (C, 4.0; water). *dl*-Nicotine monomethiodide was also prepared by the same procedure from *dl*-nicotine. M.p., 138~140°C. UV. λ_{max}^{MeOH}, 226 and 260 mμ. $[\alpha]_D^{24} = -4.08°$ (C, 4.0; water). *Anal.* Calcd. for $C_{11}H_{17}N_2I$: C, 43.43; H, 5.63; N, 9.21%. Found: C, 43.77; H, 6.03; N, 9.16%.

Insecticidal test.

The insecticidal tests against five insects were carried out by the methods mentioned below. Mortalities were recorded after keeping the treated insects for 24 hours at 26±2°C in each test. The percentages were corrected by Abbott's formula. Two repetitions for each of four different concentrations of the toxicant were averaged. The probit analysis was done for the calculation of the insecticidal activities, LD_{50} and LC_{50}. ED_{50} and EC_{50} are the median effective dose and concentration respectively, which were based on the arbitrary standard and estimated as in the insecticidal activity.

The house flies, *Musca domestica* L., were three to four days old female adults (av. 200mg/10 flies) and used for two different methods. The topical application method was the same as described in the previous paper[12]. The injection method was done by injecting 1 μl of the aqueous solution of the toxicant into the mesonotum of the house fly using a microsyringe and the treated flies (each 20 flies in a dish) were kept in a petri dish (9cm diameter×5cm height) covered with a net. The American cochroachs, *Periplaneta americana* L., were the mixed sex of the adults (av. 13.3g/10 roachs). The topical application method was done by applying 1 μl of the acetone solution of the toxicant on the neck of the roach and the treated roachs (each 5 roachs in a dish) were kept as the house flies injected. The arbitrary standards for the responses of roach were as follows, a=normal or walk with or without a poke, b=respond, but not walk by the stimulation, c=death or immobile state even after the stimulation. The apterous viviparous adults of the mealy plum aphid, *Hyalopterus arundinis* F., were collected together with a peach's leaves infested in field. The spray method was done by spraying 5m*l* of the aquous solution of the toxicant from 40cm height with the sprayer connected to the compressor (0.3 kg/cm^2) and the treated aphids (each one leaf, 90~180 aphids, in a vessel) were kept in a jelly vessel (7cm×5cm height). The rice weevils, *Sitophilus zeamais* M., were three to four days old adults with mixed sex. The dipping method was done by dipping the insects in the aquous solution

of the toxicant for one minute at 25°C and pH 10.5. After wiping off the adhered solution using a filter paper, the treated weevils (each 30 weevils in a dish) were kept in a petri dish (9cm diameter ×1cm height) covered with a filter paper. An arbitrary standard for the effectiveness was given according to the observation that insects were unable to move after repeated poke. The rice stem borers, *Chilo suppressalis* W., were the matured larva (av. 66mg/10 borers) after 40 days from hatching. The topical application method was done by treating 1 μl of the acetone solution of the toxicant on the 6-8th dorsal segment of the larvae using microsyringe and the treated borers (each 8 borers in a vessel) were kept in a jelly vessel as used for the aphids. The arbitrary standards for the responses of the borers were the same as with roachs.

Anticholinesterase assay.

The enzyme preparation and the enzyme reaction were the same as reported in the a previous paper[11]. The enzyme preparation derived from house fly head homogenate followed by the centrifugation was incubated in the phosphate buffer solution with and without the inhibitor in the presence of 2.0mM acetylcholine as a substrate at pH 7.4 and 37°C. I_{50}, which indicate the 50 percent inhibitory concentration in the enzyme reaction mixture, was estimated by the probit analysis from the percent inhibitions.

Results and Discussion

As a typical example of equitoxicity, insecticidal activities of the optical isomers of nicotine against the house fly are shown in Table 1. The summerized data (Table 2) show that *l*-nicotine is two times more toxic than *d*-nicotine against mealy plum aphid and rice stem borer. On the other hand, against house fly, American cockroach and rice weevil, both isomers show nearly the same degree of the toxicity. The interaction of acetylcholinesterase derived from house fly head with both isomers was quite different. *d*-Nicotine is 11.3 and 4.2 times more inhibitory than *l*-and *dl*-compounds, respectively. The similar tendency was also shown in the case of nicotine monomethiodide, that is, *dl*-form is 1.7 times more inhibitory than *l*-form.

In a number of cases, it is mentioned[13] that acetylcholinesterase shows stereospecificity to inhibitors. L-α-Amino acids are weak inhibitors, while D-amino acids are ineffective[14]. With the compound $(CH_3)_2N\text{-}CHCH_3\text{-}CH_2NC_5H_{10}$, the (+)-form has 4 times the inhibitory power of its antipode[15]. Also regarding to a substrate, acetyl-β-methylcholine, the(−)-form is not hydrolysed by horse serum cholinesterase, while the (+)-form and the (±)-form are hydrolysed effectively[16]. Degradation of nicotine in plant as mentioned before was also preferential to *d*-isomer. *d*-Nicotine has probably the preferential affinity to the enzyme, particularly degradative enzyme, which may reflect the difference of physiological activities, because in few case *d*-isomer exceeded *l*-isomer in toxicity as shown in Table 3.

Contrary to this, *d*-form of nornicotine was more toxic than *l*-form except for aphid and the interaction of *l*-form with house fly head acetylcholinesterase was several times more than that of *d*-form. Again, *l*-form might be more degraded than *d*-form. However, it seemed strange that the introduction of a methyl group in a position remoted from an asymmetric center caused such reverse relationship.

From varing relative toxicity of *d*- and *l*-forms of nicotine and nornicotine depending on the physiological systems for which the nicotinoids were applied, and from the structural feature associated with the insecticidal activity, Yamamoto[17] assumed that both optical isomers behave similarly at the site of action. The present authors still prefer this idea, but further biochemical and electrophysiological investigations will be needed to clarify the problem.

Summary

The toxicities of the optical isomers of nicotine were compared against the insects from five different orders. Both isomers showed almost the same toxicities against *Musca domestica* L., *Periplaneta americana* L., and *Sitophilus zeamais* M., whereas against *Hyalopterus arundinis* F. and *Chilo suppressalis* W., *l*-nicotine was about two times more toxic than *d*-isomer. As to the inhibitory power for house fly head cholinesterase, *d*-nicotine was 11.3 times stronger than *l*-isomer,

Table 1. Toxicity of the optical isomers of nicotine against *Musca domestica* L.

Compound	% Mortality at dose.												
	Topical (μg/female)				Injection (μg/female)								
	20	13	8.5	5.5	130	85	55	36	11	7.2	4.6	3.1	2.0
l-	95	70	30	15					81.3	58.4	31.3	12.5	6.3
dl-	95	80	45	10					81.3	62.5	37.5	8.7	3.1
d-	95	80	35	10					75.0	53.2	27.2	21.9	6.3
l-mono-methiodide					72.3	48.6	16.2	5.4					

Table 2. Physiological activities of *l*-, *dl*- and *d*-nicotinoids.

2-1. Toxicity to insects.

Insect	Method	Estimation value*			Regression equation***
Hyalopterus arundinis F.	Topical	LC_{50}(m%)	*l*-	24	$Y=5+2.58(X-1.380)$
			dl-	32	$Y=5+2.56(X-1.505)$
			d	48	$Y=5+2.49(X-1.681)$
Chilo suppressalis W.	Topical	LD_{50}(μg/g)	*l*-	266	$Y=5+2.62(X-2.425)$
			d-	530	$Y=5+1.99(X-2.724)$
		ED_{50}(μg/g)	*l*-	190	$Y=5+2.58(X-2.279)$
			d-	370	$Y=5+4.17(X-2.579)$
Musca dometsica L.	Topical	LD_{50}(μg/g)	*l*-	495	$Y=5+4.81(X-2.695)$
			dl-	480	$Y=5+5.28(X-2.681)$
			d-	520	$Y=5+5.78(X-2.761)$
	Injection	LD_{50}(μg/g)	*l*-	325	$Y=5+3.22(X-2.512)$
			dl-	315	$Y=5+3.95(X-2.495)$
			d-	330	$Y=5+2.89(X-2.519)$
			l-m	4,450	$Y=5+3.12(X-3.648)$
Periplaneta americana L.	Topical	LD_{50}(μg/g)	*l*-	390	$Y=5+3.76(X-2.152)$
			d-	400	$Y=5+3.49(X-2.602)$
		ED_{50}(μg/g)	*l*-	288	$Y=5+2.92(X-2.459)$
			d-	278	$Y=5+2.64(X-2.444)$
Sitophilus zeamais M.	Dipping	EC_{50}(%)	*l*-	7.4	$Y=5+2.47(X-0.857)$
			dl-	7.1	$Y=5+2.52(X-0.851)$
			d-	7.2	$Y=5+3.79(X-0.851)$

2-2. Inhibitory power of house fly head cholinesterase

Method	Estimation value			Regression equation
in vitro, in the presence of 2.0mM acetylcholine at pH 7.4 and 37°C.	I_{50}(mM)	*l*-	2.6	$Y=5+2.03(X-0.462)$
		dl-	0.62	$Y=5+1.52(X-0.181)$
		d-**	0.32	$Y=5+1.58(X-0.225)$
		d-	0.23	$Y=5+1.20(X-0.602)$
		l-m	1.0	$Y=5+1.40(X-0.041)$
		dl-m	0.64	$Y=5+1.52(X-0.181)$
		l-nornicotine	4.2	$Y=5+1.28(X-0.111)$
		dl-nornicotion	9.7	$Y=5+1.14(X-0.327)$

* The letters *l*-, *dl*-and *d*-indicate *d*-, *dl*- and *d*- nicotine, and each $[\alpha]_D^{24}$ is −168°, −0.51° and +168°, respectively. The letter *m* means monomethiodide.

** $[\alpha]_D^{24}$ is +139°.

*** X=Dose or concentration, with the units indicated for each estimation value, in logarithm. Y=Mortality or activity in probit.

Table 3. Comparison of the physiological activities of the optical isomers of nicotine and nornicotine.

3-1. Nicotine

Animal	Method of evaluation	Relative activity *l*-	*dl*-	*d*-	$[\alpha]_D$ of *d*-form	References
Invertebrate						
Protozoa	Time to kill	1.0		1.0	+140°	(18)
Coelenterata	〃	1.0		1.0	〃	〃
Plathelminthes	〃	1.0		1.0	〃	〃
Rotatoria	〃	1.0		1.0	〃	〃
Nemertini	〃	1.0		1.0	〃	〃
Annelida	〃	1.0		1/2.5	〃	〃
Chaetognatha	〃	1.0		1/2.7	〃	〃
Arthropoda						
Crustacea	〃	1.0		1.0	〃	〃
Insecta						
Gammarus	〃	1.0		1.0	〃	〃
Drosophila	〃	1.0		1.0	〃	〃
Aphis	LC_{50}	1.0	1/3			(19)
〃	〃	1.0	1/2			(20)
〃	〃	1.0		1/5		(21)
Musca	Mortality	1.0		ca. 1.0	+168°	(17)
Vertebrate						
Tadpole	Time to kill	1.0	1/2.3			(22)
〃	〃	1.0	1/2.5			(19)
〃	MLC	1.0		1/3.0	+140°	(18)
Fish	〃	1.0		1/2.7	〃	〃
Goldfish	Time to kill	1.0	1/1.5			(22)
Water turtle	〃	1.0	1/2			〃
Land turtle	〃	1.0	1.3			〃
Lizard	MLC	1.0		1/2.4	+140°	(18)
White mice	Time to kill	1.0	1/2			(22)
White rat	〃	1.0	1.2			〃
Rat	LD_{50}	1.0		ca. 1.0	+168°	(23)
〃	CD_{50} for convulsion	1.0		1/6.2	+127°	(24)
〃	50% Blocking conc. of aboidance	1.0		1/7.1	〃	〃
Guinea pig	LD	1.0		1/2	+163.2°	(6)
〃	Time to kill	1.0	1/1.2			(22)
〃	LD_{50}	1.0		ca. 1.0	+168°	(23)
Cat	LD	1.0	1/1.5			(22)
Dog	Effect on blood pressure	1.0		1/4.2	+127°	(24)
Bird	MLC	1.0		1/3.1	+140°	(18)

3-2. Nornicotine

Aphis	LC_{50}	1.0	1/2	1/1.4		(20)
〃	〃	1.0	1/1.2			(21)
Guinea pig	LD_{50}	1.0		2.8	+84°	(23)
Rat	〃	1.0	2.2	3.9	〃	〃

while *l*-nornicotine was 3.6 times than *dl*-form. Comparison of the physiological activies of *d*- and *l*-nicotinoids was reviewed. By reviewing all the available data on the physiological activities of the optical isomers of nicotinoids, it is assumed that the optical isomers behave similarly at the site of action, but differently in the process to reach the site.

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