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Note

Insecticidal and Neuroblocking Activities of Thiamethoxam-Type Compounds in the American Cockroach (*Periplaneta americana* L.)

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Insecticidal thiamethoxam and related heterocyclic compounds were examined using American cockroaches to see if they decayed to the acyclic neonicotinoid clothianidin and what was responsible for activity. The minimum insecticidal dose was 1.4 nmol for thiamethoxam, and 2.0 mmol for clothianidin, while the doses for triazine and thiadiazine analogues were evidently larger than that for clothianidin. In a physiological salt solution of thiamethoxam for 7 days, the appearance of clothianidin could not be confirmed, while the related compound triazine partly decayed to clothianidin. From these results, the prodrug concept does not necessarily apply to thiamethoxam but does to the triazine in the present experiment. Insecticidal and neuroblocking tests were also conducted for three chloronicotinyl derivatives of thiamethoxam, triazine and thiadiazine analogues, six *N*-desmethyl derivatives and three acyclic clothianidin analogues.

Keywords: prodrug, thiamethoxam, clothianidin, insecticidal potency, American cockroach.

INTRODUCTION

Successful designs of a molecule which has to be transformed in order to show or enhance the biological activity of the original structure, a so called prodrug, are found often in developed pesticides.[1,2]

The neonicotinoid thiamethoxam (**2**) may be taken as one example. This commercial insecticide shows highly effective field action especially in the control of chewing and sucking insects, common targets for imidacloprid (**14**), clothianidin (**16**) and other neonicotinoids. However it shows far less binding affinity than other neonicotinoids in housefly head membrane preparations and also in electrophysiological whole cell voltage clamp studies neurons isolated from *Heliothis virescens* ventral nerve cord showed no response to thiamethoxam when applied at a concentration of 0.3 mM.[3,4] The oxadiazine framework of thiamethoxam is constructed by the dehydrative condensation of acyclic diamine, formaldehyde and formic acid, and hence the hydrolytic regeneration of the starting components is conceivable.

Judging from its biological features and structural makeup, the diamine component of clothianidin (**16**), another neonicotinoid insecticide,[5] is suspected to be responsible for the actual insecticidal activity of thiamethoxam. In fact Nauen *et al*. revealed by LC-MS/MS analysis that thiamethoxam was rapidly metabolized to clothianidin when orally administered to *Spodoptera frugiperda* larvae or applied to cotton plants.[6]

On the other hand, Wiesner and Kaiser[3] and Maienfisch *et al*.[4] concluded that the activity of thiamethoxam was due to itself with its unique mode of binding to the target sites distinct from that of other neonicotinoids in light of a binding experiment using aphid membranes.

We have been studying the biological activity of neonicotinoid insecticides through injection and neurophysiological measurements using the American cockroach.[7–10] We examined thiamethoxam in the present study. Our study also includes triazine and thiadiazine analogues (**5**–**12**).[11–15] The prodrug concept can apply to these compounds because they are constructed in a similar fashion as thiamethoxam, *i.e*. condensation of clothianidin (or its chloropyridyl derivative) with formaldehyde and individual heteroatom sources.

MATERIALS AND METHODS

1. Materials

Imidacloprid (**14**)/clothianidin (**16**) and compounds **7**/**8** were obtained from Bayer CropScience Co. Ltd. and AgroKanesho Co. Ltd., respectively. The methods used to prepare compounds **1**–**12**,[16,17] **13**[18] and **15**–**18**[16,17] have been described together with some physical data. Here only NMR spectral data not available in the literature for compounds **6**, **8**–**10**, **12** and **13** were provided with chemical shifts in δ (ppm) and the coupling constants in Hz.

6: ^{1}H-NMR ($CDCl_3$, δ) 2.50 (3H, s), 3.06 (3H, s), 4.32 (2H, s), 4.33 (2H, s), 4.76 (2H, s), 7.50 (1H, s); ^{13}C-NMR ($CDCl_3$, δ) 35.8, 39.9, 44.3, 67.2, 70.4, 134.2, 141.1, 154.1, 156.9. **8**: ^{1}H-NMR ($CDCl_3$, δ) 2.49 (3H, s), 3.09 (3H, s), 4.24 (2H, s), 4.33 (2H, s), 4.68 (2H, s), 7.36 (1H, d, J=8.1), 7.80 (1H, dd, J=8.1/2.6), 8.82 (1H, d, J=2.6); ^{13}C-NMR ($CDCl_6$, δ) 35.6, 39.9, 48.7, 67.1, 70.4, 124.9, 129.5, 139.4, 149.2, 151.9, 157.4. **9**: ^{1}H-NMR (CD_3COCD_3, δ) 4.57 (2H, s), 4.73 (2H, s), 4.81 (2H, s), 7.71 (1H, s), 10.14 (1H, bs); ^{13}C-NMR (CD_3COCD_3, δ): 43.6, 47.9, 49.5, 136.7, 12.4, 153.0, 155.6. **10**: ^{1}H-NMR ($CDCl_3$, δ) 3.23 (3H, s), 4.44 (2H, s), 4.48 (2H, s), 4.86 (2H, s), 7.52 (1H, s);

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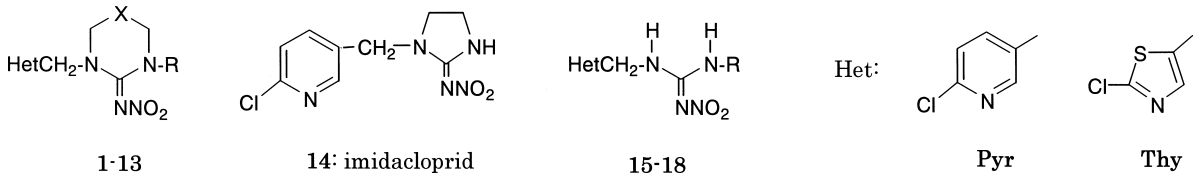

Fig. 1. Test compounds and imidacloprid.

^{13}C-NMR ($CDCl_6$, δ) 40.1, 48.0, 48.7, 51.4, 133.6, 134.9, 141.4, 157.8. **12**: ^{1}H-NMR ($CDCl_3$, δ) 3.28 (3H, s), 4.36 (2H, s), 4.51 (2H, s), 4.83 (2H, s), 7.37 (1H, d, J=8.5), 7.81 (1H, dd, J=8.5/2.0), 8.34 (1H, d, J=2.0); ^{13}C-NMR ($CDCl_6$, δ) 40.2, 48.2, 51.4, 53.0, 108.7, 125.1,139.5, 156.4, 164.8, 167.4. **13**: ^{1}H-NMR ($CDCl_3$, δ) 2.03 (2H, m), 3.37 (2H, m), 3.50 (2H, m), 4.71 (2H, s), 7.33 (1H, d, J=8.6), 7.76 (1H, dd, J=8.6/2.3), 8.30 (1H, d, J=2.3), 9.83 (1H, bs); ^{13}C-NMR ($CDCl_6$, δ) 20.1, 38.8, 45.2, 49.4, 124.6, 130.6, 139.0, 149.0, 151.3, 156.1.

2. *Biological Assays*

Reagent-grade piperonyl butoxide (PB), an inhibitor of oxidative metabolism, is commercially available. NIA16388 (propargyl propyl benzenephosphonate; NIA), an inhibitor of the hydrolytic metabolism of a pyrethroid, tetramethrin,[19] was the same sample used in previous studies.[7–10]

2.1. *Insecticidal tests on American cockroaches*

The insecticidal test on adult male American cockroaches, *Periplaneta americana* L., was conducted as described previously.[7–10] In short, a volume (1–10 μl) of a methanol solution of each compound containing some amount of dimethyl sulfoxide (DMSO) was injected into the abdomen of a cockroach unless otherwise noted. Organic solvents alone in this range did not show any toxic effect. The method of dosing was described previously in detail.[7] In some experiments, a methanol solution (1 μl) containing PB (50 μg) and NIA (50 μg) was injected 1 hr before injection of the test compounds. The metabolic inhibitors in these amounts did not have any toxic effect. To determine the minimum lethal dose (MLD in mol) for each compound, three insects were used for each dose. They were kept at 22–25°C for 24 hr after the injection. The minimum lethal dose at which two of three insects died was taken as the MLD. Paralyzed insects were counted as dead. The MLD values for the test compounds are listed in Tables 1 and 2.

2.2. *Neurophysiological test*

The neurophysiological activity of the test compounds was measured in practically the same way as that previously reported.[7–10] In brief, the excised abdominal central nerve cord of an adult male American cockroach was cut out between the fourth and fifth ganglia. One of two bundles divided from the thoracic side of the nerve cord was tightly taken up with saline (pH 7.3) into a glass tube, in which a chlorinated silver wire was set as the elec-

Table 1. Biological activities of thiamethoxam-type compounds[a)]

Compound				Insecticidal activity (MLD, nmol)[b)]			Neuroblocking activity[c)]
No.	X	Het	R	Alone	+(PB+NIA)	Synergistic effect	BC (μM)
1	O	Thy	H	12	0.59	20	6.0 (5.1–7.1)
2[d)]	O	Thy	Me	1.4	0.35	4	32 (23–38)
3	O	Pyr	H	9.2	0.60	15	20 (19–22)
4	O	Pyr	Me	14	2.3	6	23 (20–30)
5	NMe	Thy	H	180	18	10	82 (71–97)
6	NMe	Thy	Me	11	0.67	16	180 (140–230)
7	NMe	Pyr	H	280	70	4	160 (150–190)
8	NMe	Pyr	Me	98	3.1	32	630 (630–790)
9	S	Thy	H	60	1.9	3	6.5 (5.9–7.8)
10	S	Thy	Me	21	1.3	16	16 (14–18)
11	S	Pyr	H	110	1.4	79	17 (16–17)
12	S	Pyr	Me	14	2.2	6	83 (78–90)
13	CH_2	Pyr	H	6.1	0.076	80	4.5 (3.6–5.5)
14[e)]				1.1	0.071	15	2.3 (2.0–2.5)

[a)] Chemical structures are shown in Fig. 1. [b)] The value has a deviation of 0.64 to 1.6-fold. [c)] Values in parentheses are the deviation range estimated from the concentration-response relationship where each point was determined from more than three runs. [d)] Thiamethoxam. [e)] Imidacloprid; data from Ref. 8).

Table 2. Biological activities of clothianidin-type compounds[a)]

Compound			Insecticidal activity (MLD, nmol)[b)]			Neuroblocking potency[c)]
No.	Het	R	Alone	+(PB+NIA)	Synergistic effect	BC (μM)
15	Thy	H	>400	>400	—	680 (650–720)
16[d)]	Thy	Me	2.0	0.16	13	10 (8.0–10)
17	Pyr	H	>400	>400	—	>650
18	Pyr	Me	5.1	0.51	10	8.5 (7.8–9.6)

[a)] Chemical structures are shown in Fig. 1. [b,c)] See the footnote in Table 1 and the mark>means that the values are larger than those indicated. [d)] Clothianidin.

trode. As the reference electrode, another silver wire was set outside the tube. The number of spontaneous discharges that were larger than 15 μV was consecutively counted with a pulse counter (MET-1100, Nihon Kohden, Tokyo) over 30 sec periods. When the frequency decreased and stabilized within a range of 30–400 counts per 30 sec, the saline solution was exchanged for saline containing each test compound dissolved in methanol containing some amount of DMSO. The final concentration of the organic solvents was lower than 1% (v/v), which did not affect the neural activity of compounds. Experiments were conducted at 22–25°C. The concentration for each compound required to block the excitation to a certain level, BC (M), was determined from a dose-response relationship as described previously.[8–10)] The values are listed in Tables 1 and 2.

3. Hydrolysis Measurements

A three ppm SOS solution (NaCl 100 mM, KCl 2.4 mM, $CaCl_2$ 1.8 mM, and HEPES 5.0 mM, pH=7.6) of each compound was kept at 25°C and an aliquot was injected at 24, 48, 96 or 168 hr into an HPLC system (Jasco UVDECK-100-VI, 254 nm) with an ODS column (Merck, LiChrosorb RP-18) using acetonitrile/water (30 : 70, v/v) as the mobile phase. The residual amount (%) of the compound is given in Table 3.

RESULTS AND DISCUSSION

The insecticidal activity of compounds **1**–**12** injected into adult American cockroaches is listed in Table 1 along with that of a hexahydropyrimidine derivative (**13**) and imidacloprid (**14**) as references. The effect without a synergist (alone) was dependent on the heteroaromatic ring or the heterocyclic ring conjugated to the nitroimino group. Among the test compounds, thiamethoxam (**2**) stood out. Its MLD value was as low as 1.4 nmol comparable to that of imidacloprid (**14**). The insecticidal effects of compounds **1**, **3**, **4**, **6**, **10** and **12** were roughly the same as the effect of the prototype (**13**), while compounds **5**, **7**–**9** and **11** were far less active. Chlorothiazolylmethyl and chloronicotinyl groups appear to be equally effective activators as seen in the pairs **1**/**3**, **5**/**7** and **10**/**12**, except the pairs **2**/**4** and **6**/**8**, where the former group evidently excelled. As the heterocycle, an oxadiazine ring was generally more favorable than the thiadiazine and/or triazine rings (**1** *vs*. **5**/**9**; **2** *vs*. **6**/**10**; **3** *vs*. **7**/**11**; **4** *vs*. **8**). It has been known that imidacloprid and its nitromethylene analogues significantly lose the activity by methylation at *N*3 site of the imidazolidine ring,[7,20,21)] and the distorted coplanarity of the nitroguanidine (nitromethylene) moiety has been described as defecting the fit with the binding site and hence reducing the activity.[22,23)] It is of note that for the present compounds except the pair **3**/**4**, a methyl substitution at *N*3 enhanced the potency. In view of the contrast to other neonicotinoids, a different mode of binding may apply to thiamethoxam and the six-membered heterocyclic analogues tested here.

Pretreatment with synergists (PB+NIA) enhanced the activity of all compounds to various degrees. Recent studies using recombinant cytochrome P450 (CYP450) isoenzymes demonstrated that one of the principal metabolic processes for imidacloprid is reduction at the nitroimine substituent, and that concomitant oxidation at the imidazolidine moiety forming 5-hydroxy and olefin is only a minor path.[24,25)] This may explain the appreciable synergistic effects of these P450 inhibitors on all the test compounds bearing a nitroimino moiety.

To evaluate the activity at the target site, we measured the effect of the compounds on nerve impulses in a saline solution. Neonicotinoid compounds immediately increase the frequency of impulses in nerve preparations, after which there is a drop to a

Table 3. Residual composition in an SOS solution of **2**, **4**, **6**, **8**, **10** and **12** at 25°C at the given hours[a)]

Compound (%)	Time (hr)				
	0	24	48	96	168
2	100	99	97	98	97
4	100	99	97	96	96
6/**16**	100/0	88/8	79/12	66/32	58/39
8/**18**	100/0	86/11	77/20	63/35	46/50
10	100	96	87	79	70
12	100	95	84	80	69

[a)] See the text for measurement conditions. The data are from one run.

level lower than the control. For a quantitative analysis of the structure-activity relationship including the hydrophobic parameter, the strength of the neuroblocking activity is a more reliable indicator than the nerve excitation effects.[8,10] All the compounds tested here blocked nerve impulses at concentrations between 4 and 630 μM (Table 1). There are a few other remarks in the tests. First, the *N*3-H derivatives had stronger neuroblocking activity than the *N*3-Me derivatives. This tendency is along with imidacloprid and its analogues, conferring a considerable drop in methylation at the corresponding site of the imidazolidine ring.[7,8] Second, the triazines were notably less active than the oxadiazines and the thiadiazines. We ascribe the weak neuroblocking activity of the triazine compounds (**5**–**8**) to the ionization of the *N*5 nitrogen atom in the saline solution.

The biological tests of clothianidin (**16**) and the analogous acyclic compounds revealed a distinct difference in activity between *N*3-Me and desmethyl derivatives. Regardless of the attached heteroaromatic ring, the NH compounds (**15**, **17**) were inactive in the insecticidal as well as nerve tests, while the methyl compounds **16** and **18** were highly active in both tests. These results suggest that a lipophilic entity at this site is indispensable for these acyclic neonicotinoids to exhibit activity.

To examine the hydrolytic stability in the outside milieu, we measured the residual amounts of oxadiazines **2** and **4**, triazines **6** and **8**, and thiadiazines **10** and **12** in a physiological salt solution at 25°C (SOS solution, pH 7.6). Triazines **6** and **8** decayed about 10% to **16** and **18** after 24 hr, while we could not detect any measurable amount of the acyclic compounds from oxadiazines **2** and **4** even after 7 days under these conditions. The decomposition of thiadiazines **10** and **12** was observed, but the ratios of acyclic molecules could not be determined because several decomposed components overlapped in the area of the peak due to the acyclic compound. The sluggish decomposition rates of the oxadiazine compounds compared to the other heterocycles can be ascribed to the larger bond energy for C–O (85–91) than for C–N (69–75) and for C–S (61) bonds in kcal/mol.[26]

The significantly weak affinity for the [^{3}H]imidacloprid binding sites on insect nAChR from housefly head membrane and absence of pulses in neurons isolated from a lepidopteran species have been the basis for considering thiamethoxam as a prodrug.[6] The present experiment revealed that although its neuroblocking potency and insecticidal activity with synergists, *i.e*. under the conditions where the effects of metabolic decomposition were limited, were inferior to those for clothianidin, thiamethoxam matched clothianidin in insecticidal activity without synergists. From the results of matching insecticidal activity (alone), there seems no need to consider the prodrug possibility in the present case using American cockroaches.

Triazine compounds **6** and **8** appear different. Their neuroblocking effects were far inferior to those of **16** and **18**, but their insecticidal activities were not much weaker. Basic molecules like triazines are ionized in the fluids of insects and reach the synapses only slowly through the lipophilic cuticles and the ion barriers. During retarded movement, the molecule is prone to decompose. This tendency is clearer on estimation of the insecticidal activity 24 hr after injection than estimation of the neuroblocking action at 10 min. We suspect that partial hydrolysis, enzymatically and nonenzymatically, took place and generated nonionizable acyclic molecules that contributed to the insecticidal activity of compounds **6** and **8**.

The thiadiazine derivatives showed another biological feature. The insecticidal activities with synergists were very similar for NH (**9**/**11**) and N–Me (**10**/**12**) compounds as opposed to the other heterocycles. This means that the common framework of Het–CH_2NCH_2–S–CH_2NR is a target for metabolism and the scission of the labile C–S bonds occurs in several ways, giving various metabolites having different levels of activity and a balance appeared as insecticidal potency without synergist treatment.

We have discussed the biological effect of three quite similar compounds and learned that even a minor structural change, in this case only a single heterorings atom, can bring about a remarkable difference in biological behavior, which suggests that molecular design based on a prodrug concept is not always accord with a prediction.

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REFERENCES

1) J. Drabek and R. Neuman: "Progress in Pesticide Biochemistry and Toxicology," ed. by D. H. Hutson and T. R. Roberts, Wiley, New York, pp. 21–26, 1985.
2) S. Kagabu: "Chemistry of Crop Protection," ed. by G. Voss and G. Ramos, Wiley-VCH, Weinheim, pp. 193–212, 2003.
3) P. Wiesner and H. Kayser: *J. Biochem. Molec. Toxicol.* **14**, 221–230 (2000).
4) P. Maienfisch, M. Angst, F. Brandl, W. Fischer, D. Hofer, H. Kayser, W. Kobel, A. Rindlisbacher, R. Senn, A. Steinemann and H. Widmer: *Pest Manag. Sci.* **57**, 906–913 (2001).
5) H. Uneme, K. Iwanaga, H. Higuchi, Y. Kando, T. Okauchi, A. Akayama and I. Minamida: *9th IUPAC Conf. On Pestic. Chem.*, *Abstr.* **1**: 1D–009, 1998.
6) R. Nauen, U. Ebbinghaus-Kintscher, V. L. Salgado and M. Kaussmann: *Pestic. Biochem. Physiol.* **76**, 55–69 (2003).
7) K. Nishimura, Y. Kanda, A. Okazawa and T. Ueno: *Pestic. Biochem. Physiol.* **50**, 51–59 (1994).
8) K. Kiriyama, K. Iwaya, S. Kagabu and K. Nishimura: *J. Pestic. Sci.* **26**, 55–59 (2001).
9) S. Kagabu, A. Azuma and K. Nishimura: *J. Pestic. Sci.* **27**, 267–271 (2002).
10) K. Kiriyama and K. Nishimura: *Pest Manag. Sci.* **58**, 669–676 (2002).
11) K. Shiokawa, S. Tsuboi, K. Moriya, Y. Hattori, I. Honda and K. Shibuya (Nihon Tokushu Noyaku Seizo Co., Ltd.): *Eur. Pat. Appl.* EP 386565 A1 (1990).
12) K. Ishimitsu, J. Suzuki, T. Kishimoto, H. Ohishi, T. Yamada, R. Hatano and N. Takakusa (Nippon Soda Co., Ltd.): *PCT. Int. Appl.* WO91-1978 A1 (1990).

13) K. Nanjo, K. Takasuka, S. Segami and A. Kariya (AgroKanesho Co., Ltd.): *Eur. Pat. Appl.* EP 425978 A2 (1991).
14) H. Uneme, K. Iwanaga, N. Higuchi, I. Minamida and T. Okauchi (Takeda Chem. Co., Ltd.): *Eur. Pat. Appl.* EP 376279 A2 (1991).
15) O. Kristiansen, P. Maienfisch and L. Gsell (Ciba-Geigy AG): *Eur. Pat. Appl.* EP 418199 A2 (1991).
16) P. Maienfisch, H. Huerlimann and J. Haettenschweiler: *Tetrahedron Lett.* **41**, 7187–7191 (2000).
17) P. Maienfisch, H. Huerlimann, A. Rindlisbacher, L. Gsell, H. Dettwiller, J. Haettenschweiler, E. Sieger and M. Walti: *Pest Manag. Sci.* **57**, 165–176 (2001).
18) K. Shiokawa, S. Tsuboi, S. Kagabu and K. Moriya (Nihon Tokushu Noyaku Seizo Co., Ltd.): *Eur. Pat. Appl.* EP 192060 (1986).
19) T. Suzuki and J. Miyamoto: *Pestic. Biochem. Physiol.* **4**, 86–97 (1974).
20) K. Nishimura, M. Tanaka, K. Iwaya and S. Kagabu: *Pestic. Biochem. Physiol.* **62**, 172–178 (1998).
21) H. Nishiwaki, Y. Nakagawa, T. Ueno, S. Kagabu and K. Nishimura: *Pest Manag. Sci.* **57**, 810–814 (2001).
22) S. Kagabu and H. Matsuno: *J. Agric. Food Chem.* **45**, 276–281 (1997).
23) M. Tomizawa, N. Zhang, K. A. Durkin, M. M. Olmstead and J. E. Casida: *Biochemistry* **42**, 7819–7827 (2003).
24) D. A. Schulz-Jander and J. E. Casida: *Toxic. Lett.* **132**, 65–70 (2002).
25) D. A. Schulz-Jander, W. M. Leimkuehler and J. E. Casida: *Chem. Res. Toxic.* **15**, 1158–1165 (2002).
26) M. B. Smith and J. March: "March's Advanced Organic Chemistry," 5th Ed., Wiley, New York, Chap. 1, 2001.