J. Pestic. Sci., **30**(1), 44–46 (2005)

Note

Insecticidal and Neuroblocking Activities in the American Cockroach (*Periplaneta americana* L.) of Mannich Bases of Nitromethylene Imidazolidine Neonicotinoids

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(Received July 14, 2004; Accepted September 30, 2004)

The insecticidal and neuroblocking activities of Mannich bases prepared from chloronicotinyl- and chlorothiazolylmethyl-nitromethylenimidazolidines, known potent insecticides, were measured in American cockroaches. The concentrations needed to cause neuroblocking in excised central nerve cord of the insects (BC) were 100–140 μM, far higher than those, 1.6–1.9 μM, for the starting compounds. However, the minimum lethal doses by injection (MLD) for the Mannich bases were 0.92–1.2 nmol, not very different from the values, 0.28–0.46 nmol, for the starting compounds. The half-life of the Mannich base decaying to the original molecule was 5.3 hr in a physiological solution, which also suggests the potential of Mannich bases as proinsecticides for nitromethylene molecules.

Keywords: proinsecticide, nitromethylene insecticide, Mannich adduct, neuroblocking potency, American cockroach.

INTRODUCTION

The dehydrative condensation of a compound containing an active hydrogen and formaldehyde with ammonia or primary or secondary amine is known as the Mannich reaction.[1] This reaction is mechanistically reversible and the condensation product called a Mannich base can be decomposed into the three starting components under hydrolytic conditions. If one starting component is a biologically active ingredient, the active substance can be regenerated *in vivo* or *in vitro* and displays activity at an appropriate stage. Such a compound is called a prodrug. In the neonicotinoid field, the known potent insecticidal nitromethylene compound **1**[2,3] or **2**[4] has served as a model for proinsecticides. Mannich bases like **3** or **4** are just such a case.[5,6] The adduct was constructed literally according to a Mannich reaction protocol, that is, the condensation of active-hydrogen bearing compound **1** or **2**, formaldehyde and methylamine.

We examined the insecticidal activity of prodrug compounds **3** and **4** on injection into cockroaches and conducted neuroblocking measurements with excised nerve preparations of this insect.[7–10]

MATERIALS AND METHODS

1. Materials

The procedures used to prepare compounds **1**,[2,3] **2**,[4] **3/4**[5] and **5**[11] are described together with some physical data in the literature. Here, NMR spectral data for compounds **2–4** lacking in the literature were reported along with chemical shifts in δ (ppm) and the coupling constant in Hz.

2: ^{1}H-NMR (DMSO-d_6, δ) 3.50–3.62 (4H, m), 4.68 (2H, s), 6.86 (1H, s), 7.70 (1H, s), 8.92 (1H, bs); ^{13}C-NMR (DMSO-d_6, δ) 41.0, 42.6, 47.5, 96.1, 135.7, 141.3, 150.8, 158.3. **3:** ^{1}H-NMR ($CDCl_3$, δ) 2.46 (3H, s), 3.59–3.77 (4H, m), 3.77 (2H, s), 3.94 (2H, s), 4.84 (2H, s), 7.34 (1H, d, J=8.4), 7.86 (1H, dd, J=8.4/2.2), 8.36 (1H, d, J=2.2); ^{13}C-NMR ($CDCl_3$, δ) 41.2, 46.8, 49.2, 52.1, 53.1, 67.1, 103.0, 124.4, 130.8, 139.3, 149.1, 151.1, 157.1. **4:** ^{1}H-NMR ($CDCl_3$, δ) 2.46 (3H, s), 3.67 (2H, bs), 3.78 (2H, s), 3.92 (2H, s), 4.93 (2H, s), 7.47 (1H, s); ^{13}C-NMR ($CDCl_3$, δ) 41.3, 46.6, 48.2, 49.0, 53.0, 67.1, 94.4, 135.2, 140.6, 154.1, 156.5.

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,[12] was the same sample used in previous studies.[7–10]

2.1. Insecticidal tests against American cockroaches

The insecticidal test against adult male American cockroaches, *Periplaneta americana* L., was conducted as described previously.[7–10] In short, various volumes (1–10 μl) of the methanol solution of each compound containing some amount of dimethyl sulfoxide (DMSO) were injected into the abdomen of the 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 show 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 24–27°C for 24 hr after the injection. The minimum dose at which two of the three insects died was taken as the MLD. Paralyzed insects were counted as dead. The MLD values for the test compounds are listed in Table 1.

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2.2. *Neurophysiological test*

The neurophysiological activity of the test compounds was measured practically in the same way as that previously reported.[7–10] In brief, the abdominal central nerve cord of an adult male American cockroach was excised 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 (210.4 mM of NaCl, 2.9 mM of KCl, 1.8 mM of $CaCl_2$, 1.8 mM of Na_2HPO_4 and 0.2 mM of KH_2PO_4; pH 7.3) into a glass tube, in which a chlorinated silver wire was set as the electrode. As the reference electrode, another silver wire was set outside the tube. The number of spontaneous discharges that were larger than 15 μV was counted consecutively with a pulse counter (MET-1100, Nihon Kohden, Tokyo) over 30-sec periods. When the frequency decreased and stabilized within a range of 30–300 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 the 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 Table 1.

RESULTS AND DISCUSSION

The insecticidal potencies of the original insecticides **1** and **2** and prodrug compounds **3** and **4** in adult American cockroaches on injection without synergists were tabled. The potencies of a compound substituted at the nitrogen atom on the imidazolidine ring of compound **1**, 3-*N*-methyl derivative **5**,[8] were given as the reference. It is noticeable that the activities of prodrugs **3** and **4** dropped by only 1/4 to 1/2 relative to those of the original compounds. This modest decrease is a good contrast to the drop to 1/60 of *N*-methyl derivative **5** relative to unsubstituted **1**.

Next we examined the behavior when pretreated with synergists PB and NIA. One of us applied these synergists to chloronicotinyl molecules and confirmed that they work on this class of insecticide.[7] Later we found that the synergistic magnitude varied with the alkyl chain length in the case of 3*N*-alkylated neonicotinoids.[8,13] Recently, Nishiwaki *et al*. gave evidence that one of the functions of PB and NIA was to interfere with enzymatic hydroxylation at the methylene of the imidazolidine ring of imidacloprid.[14] We compared imidacloprid with some of the metabolites, and found the insecticidal activity following injection without the synergists of the 5-hydroxy metabolite and the subsequently dehydrated olefin to be 1/10 or less of that of imidacloprid.[15] The principal enzyme system involved in such biological functions is the P450 system. P450 exists ubiquitously in organisms, and the prevailing mechanism is hydroxylation at the α-

Table 1. Biological activities of Mannich adducts and imidazolidine derivatives[a]

Compound No.	Insecticidal activity (MLD, nmol)[b]			Neuroblocking potency[c]
	Alone	+(PB+NIA)	Synergistic effect	BC (μM)
1	0.28	0.056	5	1.6 (1.4–1.9)
2	0.46	0.057	8	1.9 (1.4–2.6)
3	1.2	0.12	10	140 (130–150)
4	0.92	0.15	6	100 (96–110)
5[d]	17	8.5	2	140 (110–180)

[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 a dose-response relationship where each point was determined from more than three runs. [d] Data from Ref. 8.

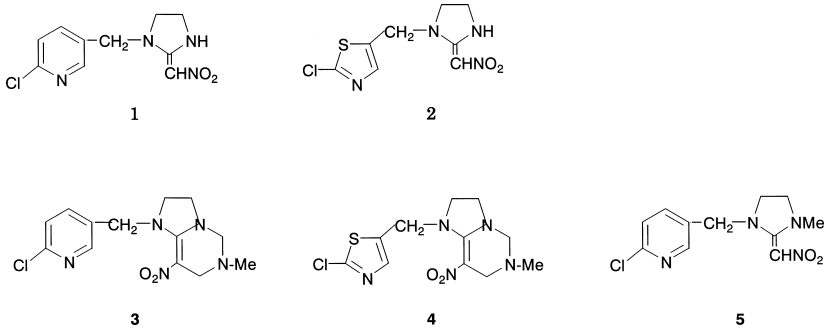

Fig. 1. Tested molecules.

methylene to the amine nitrogen atom.[16–18)]

Synergists PB and NIA exerted positive effects on all the compounds tested in a magnitude range from 2 to 10 (Table 1). It follows undoubtedly from the above discussion that the pretreated synergists were to some extent contributing to the enhancement of the activity of amine compounds **1–5** by inhibiting the enzymatic hydroxylation on the imidazolidine ring. However, the question arises as to why the synergistic effects were positive for the Mannich bases although a rather marked enhancement of potency would be expected unless the synergists hampered the release of the more potent nitromethylene molecules **1** and **2**. Recently, Casida and the coworkers carried out an informative experiment to explain this phenomenon.[19,20)] The principal process of metabolism by recombinant cytochrome P450 (CYP450) isozymes of imidacloprid is reduction at the nitroimine substituent, while the concomitant oxidation at the imidazolidine moiety forming 5-hydroxy followed by olefination is only a minor pathway. For now we are looking at the synergistic values for the prodrugs as an indication of the balance of increasing and decreasing effects caused by the synergists.

To evaluate the activity at the supposed target site, we measured the effect on the nerves of the compounds using an electrophysiological method. Neonicotinoid compounds immediately increase the frequency in nerve preparations and this increase is followed by a drop to a level lower than the control, where the strength of the neuroblocking activity is more reliable indication of the effects of test compounds.[8–10)] In the present experiment using American cockroaches there were remarkable differences in neuroblocking potency between the unmodified imidazolidines **1** and **2**, and prodrugs **3** and **4** and *N*-methyl derivative **5**. The BC values were more than 100 μM for **3–5**, far larger than the values, 1.6–1.9 μM, for **1** and **2**. The contrast with the relatively high insecticidal activity of compounds **3** and **4** on injection without the synergists is a good indication for the prodrug, which was not the case for compound **5** showing evidently lower insecticidal activity. To confirm the prodrug possibilities of compound **3** as a Mannich base, we examined its stability in the physiological salt solution used for neurophysiological tests. Compound **3** decayed to **1** with a half-life of 5.3 ± 0.2 hr (n=3) at 25°C, supporting the regeneration of the unveiled compound 24 hr after the injection.

The concentrations needed to inhibit [^{3}H]imidacloprid from binding to the *Musca* nAChR by 50% were reported to be 0.24 nM for **1** and 0.7 nM for **3**.[21)] These values are similar, whereas their blocking concentrations determined in the present study differed by two orders of magnitude (Table 1). One explanation for the distinction would be the difference in the insect species tested. Another reason may be more fundamental, the experimental conditions. As described above, neonicotinoids including these two compounds induced excitation in a shorter treatment period or at lower concentrations in the electrophysiological experiments, whereas they caused conduction blockage after longer treatment or at higher concentrations.[7–10)] Since the neurophysiological symptoms should be induced after the binding of the compounds with receptors, the excitative activity of these compounds may be related to the binding activity as the first symptom in the electrophysiological experiments.

In short, the results from insecticidal experiments and neuroblocking measurements using American cockroaches revealed a considerable contribution by regenerated **1** and **2** to the insecticidal activity on injection without synergists.

ACKNOWLEDGMENTS

We wish to thank Earth Chemical Co., Ltd. for the donation of American cockroaches.

REFERENCES

1) F. F. Blicke: “Organic Reactions,” Vol. 1, ed. by R. Adams, Wiley, New York, pp. 303–341, 1942.
2) K. Shiokawa, S. Tsuboi, S. Kagabu and K. Moriya (Nihon Tokushu Noyaku Seizo Co., Ltd.): *Eur. Pat. Appl*. 163, 855 A1 (1985).
3) S. Kagabu, K. Moriya, K. Shibuya, Y. Hattori, S. Tsuboi and K. Shiokawa: *Biosci. Biotechnol. Biochem*. **56**, 362–363 (1992).
4) K. Shiokawa, S. Tsuboi, S. Kagabu and K. Moriya (Nihon Tokushu Noyaku Seizo Co., Ltd.): *Eur. Pat. Appl*. 0,192,060 A1 (1986).
5) H. Kishida, N. Sakamoto, K. Umeda and H. Fujimoto (Sumitomo Chem. Co., Ltd.): JP 04,173,788 (1992).
6) B.-W. Krueger, H. Uhr, J. Kanellakopulos, E. R. F. Wolf, A. Turberg, N. Mencke, C. Erdelen, U. Wachendorff-Neumann and J. Harwig (Bayer AG): DE 4,236,204 A1 (1994).
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) S. Kagabu, K. Yokoyama, K. Iwaya and M. Tanaka: *Biosci. Biotechnol. Biochem*. **62**, 1216–1224 (1998).
12) Suzuki and J. Miyamoto: *Pestic. Biochem. Physiol*. **4**, 86–97 (1974).
13) K. Nishimura, M. Tanaka, K. Iwaya and S. Kagabu: *Pestic. Biochem. Physiol*. **62**, 172–178 (1998).
14) H. Nishiwaki, K. Sato, Y. Nakagawa, M. Miyashita and H. Miyagawa: *J. Pestic. Sci*. **29**, 110–116 (2004).
15) S. Kagabu, C. Kato and K. Nishimura: *J. Pestic. Sci*. **29**, 376–379 (2004).
16) T. Ohmura, T. Ishimura and Y. Fujii (eds): “Molucular Biology of P450,” Kodansha, Tokyo, 2003 (in Japanese).
17) J. Puidoux, R. Moreau and L. Gourdoux: *Biogen. Amines* **8**, 391–400 (1992).
18) R. Kaufman and D. Sloley: *Insect Biochem. Molec. Biol*. **26**, 101–109 (1996).
19) D. A. Schulz-Jander and J. E. Casida: *Toxic. Lett*. **132**, 65–70 (2002).
20) D. A. Schulz-Jander, W. M. Leimkuehler and J. E. Casida: *Chem. Res. Toxic*. **15**, 1158–1165 (2002).
21) B. Latli, M. Tomizawa and J. E. Casida: *Bioconjugate Chem*. **8**, 7–14 (1997).