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Note

Effect of Tropine Derivatives, Antimuscarinic Agents, on the Growth of *Bombyx mori* Larvae

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A number of atropine analogs were synthesized and their effects on larval growth of the silkworm, *Bombyx mori*, were investigated by both topical application and dietary administration. Among the tested compounds, 8-methyl-8-azabicyclo[3.2.1]octan-3α-ol 2,2-diphenylpropionate (5), an antagonist of the muscarinic acetylcholine receptor in mammals, significantly prolonged the duration of the instar. When fed on compound 5 at 30 ppm, some of the larvae failed to molt. A 2,2-diphenylpropionate moiety was indispensable for this activity. Compound 5 had more potent activity than atropine which is known to inhibit PTTH release *in vitro*.

Key words: atropine; tropine derivatives; antimuscarinic agent

Molting and metamorphosis in insects are regulated by several hormones including ecdysteroids, juvenile hormones and the prothoracicotroic hormone (PTTH). PTTH is a neuropeptide which is secreted from the brain and stimulates the prothoracic gland to synthesize and release ecdysteroids which initiate molting. Shirai *et al.* have recently found that PTTH release in the silkworm, *Bombyx mori*, was associated with the muscarinic acetylcholine receptor (mAChR),[1,2] resulting in specific stimulation of PTTH release with muscarine and its inhibition with the muscarinic antagonist, atropine.

Many studies with ligand-binding assays and molecular cloning techniques have shown the muscarinic receptors in mammals to be classified into at least five subtypes. Structure-activity relationship studies of muscarinic antagonists have indicated that such key functional groups as a nitrogen atom, an ester group and a bulky hydrophobic portion were required in a molecule to attach antimuscarinic properties.[3–5] Among the muscarinic antagonists, atropine is known as a non-selective antagonist for receptor subtypes. These facts prompted us to synthesize atropine analogs, in which a tropic acid moiety was replaced by other acids, and to evaluate their effects on larval growth of the silkworm. In the present paper, we report the activity of tropine derivatives as antimuscarinic agents to cause prolongation of the larval period or delayed toxicity.

Bombyx mori (Shunrei × Shogetu strain) larvae were reared on an artificial diet as previously described.[6] Atropine analogs were synthesized from the corresponding acyl chloride and tropine in toluene or benzene with triethylamine as a base.[7] The structures of the test compounds are described in Fig. 1.

When topically applied to newly molted 4th instar larvae of *B. mori* (160 μg/larvae), none of the tropine derivatives (atropine and compounds **1–6**) showed any significant effect on larval growth or molting, resulting in molting into the 5th instar similar to that by the control (data not shown). However, a daily topical application of atropine and compound **5** at 100 μg/larva slightly prolonged the duration of the instar and delayed the 4th ecdysis (Fig. 2). In the control, the 4th larval period was 4.3±0.48 days on average, while the atropine and compound **5** treatment resulted in 4.7±0.67 and 5.3±0.82 days, respectively.

Since only atropine and compound **5** showed weak activity by daily topical application, we examined the effects of atropine and compounds **5** and **6** by dietary administration in further tests (Fig. 3). Atropine at 10 and 30 ppm delayed the 4th ecdysis by 1 to 2 days compared with the control period. When fed on an artificial diet containing compound **5** at 10 or 30 ppm, the 4th larval period was significantly prolonged. At a dose of 30 ppm, 40% of the larvae lasted more than twice as long as the control insects and died without molting to the 5th instar (asterisks). It is unlikely that compound **5** had anti-feeding activity, because there was no significant difference in the quantities of excrement between the control insects and larvae treated with atropine and compound **5**. 3,3-Diphenylpropionic acid ester **6** had no influence on larval growth, even at as high a concentration as 100 ppm, indicating that the position of the phenyl group on the propionic acid moiety was important for the activity. Since tropine derivatives can be hydrolytically cleaved to the corresponding acid and tropine, we examined the effect of 2,2-diphenylpropionic acid on larval growth. It showed no activity at 100 ppm (data not shown), suggesting that compound **5** itself was responsible for the activity.

A number of antimuscarinic compounds containing a 2,2-diphenylpropionate moiety have been reported in mammals.[8,9] A study on mammalian neuroblastoma cells has shown compound **5** to have antimuscarinic activity comparable to that of atropine.[10] In the present study, compound **5** caused prolongation of the larval period and inhibited ecdysis, showing higher activity than atropine which inhibited PTTH secretion *in vitro*.

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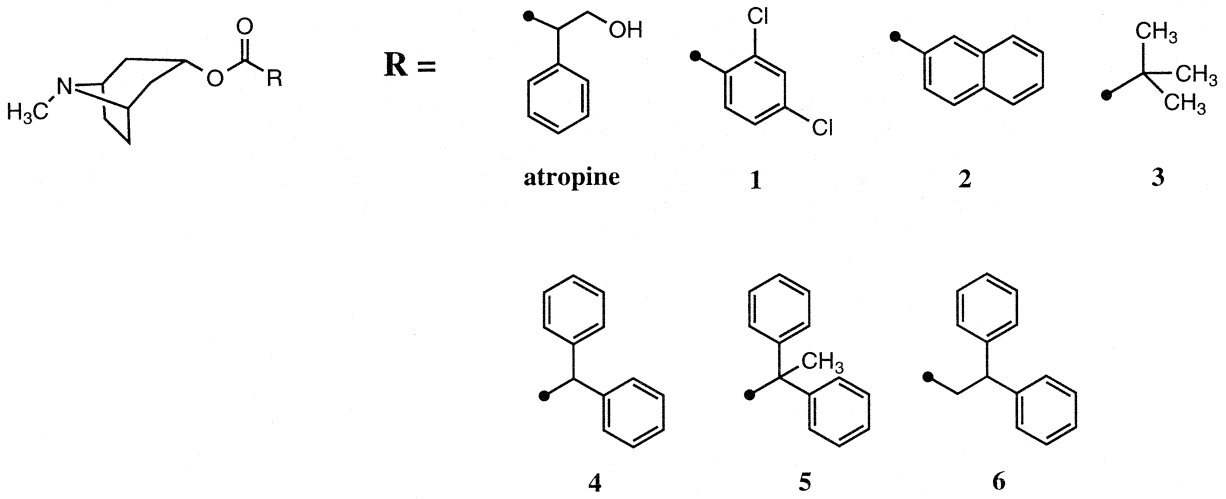

Fig. 1. Structures of Atropine and Synthesized Tropine Derivatives.

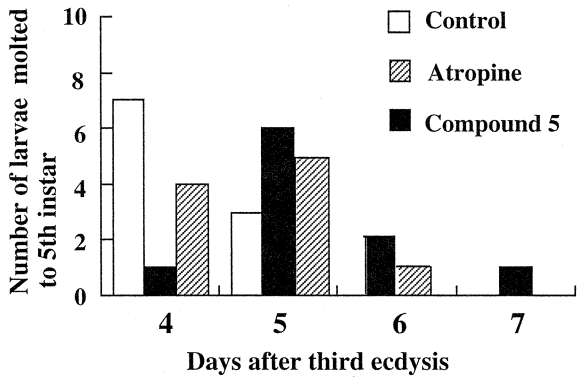

Fig. 2. Effects of Topically Applied Atropine and Compound **5** on the Growth of 4th Instar Larvae of the Silkworm.

A test compound (100 μg/larva) in an acetone solution was topically applied to newly molted 4th instar larvae of the silkworm. The larvae were subsequently treated with the test solution at 24 h intervals during the 4th larval period. Ten larvae were used for each treatment.

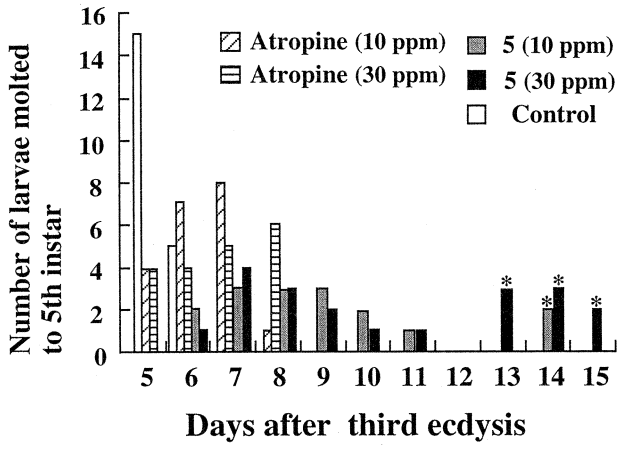

Fig. 3. Effects of Atropine and Compound **5** Administered in the Diet on the Growth of 4th Instar Larvae of the Silkworm.

A test compound was mixed with the artificial diet at a concentration of 10 or 30 ppm. The diet containing the test compound was administered through the 4th larval period. Twenty larvae were used for each dose. Asterisks indicate larvae which were not able to molt to the 5th instar and died.

Although it is not clear whether compound **5** acted on mAChRs in the silkworm, which have never been identified so far, based on the specific activity against silkworm larvae *in vivo* and on the antimuscarinic activity in mammals, it is reasonable to postulate that compound **5** may block mAChRs to suppress PTTH release. Muscarinic receptors in insects might be an unexploited target for new insect growth regulators.

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7) Compound **5**: NMR ($CDCl_3$) δ: 1.31–1.34 (2H, m), 1.54 (1H, s), 1.58 (1H, s), 1.70–1.73 (2H, m), 1.94 (3H, s), 2.06–2.08 (1H, m), 2,10–2,12 (1H, m), 2.19 (3H, s), 2.95 (2H, s), 5.07–5.11 (1H, m), 7.22–7.33 (10H, m). *Anal.* Found: C, 64.38; H, 6.61; N, 2.77%. Calcd. for $C_{27}H_{33}NO_8$ (tartrate): C, 64.92; H, 6.66; N, 2.80%.
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