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Note

Synthesis of 2,2-Diphenylpropionate Derivatives and Their Effects on Larval Growth of the Silkworm

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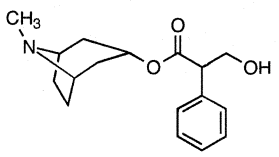
A variety of 2,2-diphenylpropionate derivatives with an amino substituent were synthesized and their effects on larval growth of the silkworm, *Bombyx mori*, were examined by dietary administration. Of the compounds tested, 3-(4-ethylpiperazin-1-yl)propyl 2,2-diphenylpropionate (3) caused significant prolongation of the larval period. Studies on the structure-activity relationship indicate that a piperazine ring and the bond distances between the nitrogen atom and the ester group were important for this activity. Treatment of compound 3 delayed the increase of ecdysteroid titers in the hemolymph by 3 days compared with that of the control, which correlates with the delay in molting.

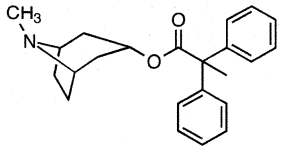
Key words: 2,2-diphenylpropionates; ecdysteroids; antimuscarinic agent

The prothoracicotropic hormone (PTTH), a neuropeptide released from the neurosecretory cells in the insect brain, stimulates the prothoracic glands to synthesize and secrete ecdysteroids which elicit ecdysis and morphological change. The PTTH release mechanism has already been partly clarified. Shirai *et al.* have indicated that PTTH release in the silkworm, *Bombyx mori*, was associated with the muscarinic acetylcholine receptor (mAChR), and atropine, a mammalian mAChR antagonist, suppressed the release of PTTH.[1] On the basis of experiments with specific inhibitors and antagonists, Aizono and Shirai have demonstrated the predictable signal transduction involved in PTTH release.[2]

We have previously reported that atropine and an atropine analog, 8-methyl-8-azabicyclo[3.2.1]octan-3α-ol 2,2-diphenylpropionate (**1**), caused growth-delaying activity against the 4th instar larvae of the silkworm by a dietary administration assay,[3] and that compound **1**, which has been shown to have antimuscarinic activity comparable to that of atropine in a mammalian neuroblastoma binding assay,[4]



atropine



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was markedly more potent than atropine in our *in vivo* assay. From a preliminary study of the structure-activity relationship, a 2,2-diphenylpropionate moiety was essential for this activity. This fact prompted us to synthesize compound **1** analogs, in which a tropine moiety was replaced by a variety of amines, and to evaluate their effects on larval growth of the silkworm. In this paper, we report the structure-activity relationships of 2,2-diphenylpropionate derivatives and the effect of a representative compound, 3-(4-ethylpiperazin-1-yl)propyl 2,2-diphenylpropionate (**3**), on the ecdysteroid titers in the silkworm larvae.

2,2-Diphenylpropionates with an amino group were prepared as shown in Fig. 1. The following procedure for the preparation of compound **3** is typical. A mixture of 1,3-dibromopropane (4.5 ml), 2,2-diphenylpropionic acid (1.5 g) and potassium bicarbonate (3.7 g) in DMF was stirred for 7 hr at room temperature. Normal workup and purification by silica gel column chromatography (3:1 hexane-ethyl acetate) gave 2.4 g of crude 3-bromopropyl 2,2-diphenylpropionate. A mixture of this compound (1.0 g), 1-ethylpiperazine (0.4 ml) and potassium bicarbonate (1.2 g) in acetonitrile was refluxed for 6 hr. After cooling, to the mixture was added water (10 ml), and the product was extracted 5 times with ethyl acetate. The combined organic layer was washed with water, dried over Na_2SO_4, and concentrated. To the resulting residue was added ether and an aqueous oxalic acid solution. The aqueous layer

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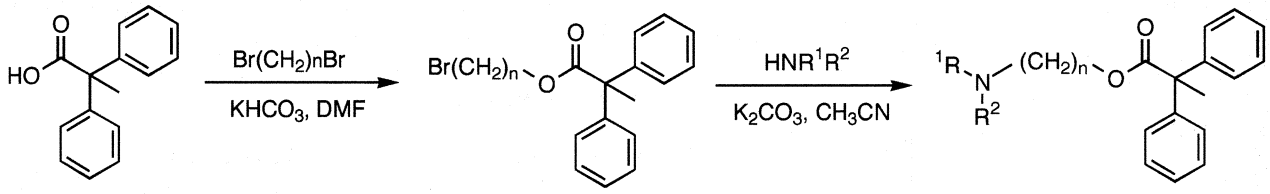
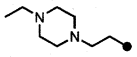
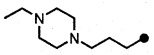
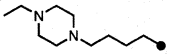
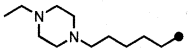
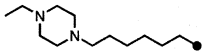
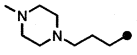
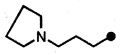
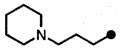
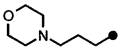
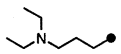
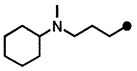


Fig. 1. Synthetic Scheme for Preparation of the 2,2-Diphenylpropionate Derivatives.

Table 1. Effects of 2,2-Diphenylpropionate Derivatives on the Growth of 4th Instar Larvae of *Bombyx mori*.

No.	R	Concentration (ppm)	Days of 4th instar[a] 30	100
2			5.1±0.5	6.2±0.5[b]
3			6.5±0.7[b]	7.2±0.8[b]
4			5.6±0.6	6.1±0.6[b]
5			5.1±0.7	5.8±0.6
6			4.7±0.6	4.5±0.6
7			6.9±0.6[b]	7.5±0.7[b]
8			5.2±0.3	6.0±0.4[b]
9			5.1±0.3	7.4±1.1[b]
10			5.0±0.0	6.1±0.3[b]
11			5.1±0.3	5.2±0.6
12			5.0±0.2	5.6±0.5
	Atropine		5.2±0.4	6.3±0.7[b]
	Compound **1**		6.1±0.4[b]	NT
	Control		5.1±0.2	

[a] Each value represents the mean±SD.
[b] Significant difference from the control value ($p<0.05$).
NT: not tested.

was separated, washed 2 times with ether, and rendered alkaline with K_2CO_3. The product was extracted with ethyl acetate, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography on neutral alumina by eluting with ethyl acetate-hexane (1:1) to give 0.57g (52%) of compound **3** as an oil.[5]

B. mori (Shunrei×Shogetsu strain) larvae were reared on an artificial diet, and a test compound in an acetone solution was mixed into the diet in the same manner as previously described.[3] The diet containing the test compound was administered throughout the 4th larval period. Twenty larvae were used for each dose.

Table 1 shows the effects of a number of 2,2-diphenylpropionates on the growth of 4th instar larvae of *B. mori*. None of the compounds tested inhibited molting into the 5th instar. 2-(4-Ethylpiperazin-1-yl)ethyl 2,2-diphenyl propionate (**2**) as well as atropine at the concentration of 100 ppm delayed the 4th ecdysis by 1 to 2 days when compared with the control period. In this (4-ethylpiperazin-1-yl)alkyl series of compounds, varying the length of the carbon chain between the nitrogen atom and the ester group significantly affected their activity. Among them, propyl analog **3** showed the highest activity, having the same activity as that of compound **1**. The activity decreased with increasing length of the alkyl chain; the pentyl (**5**) and hexyl (**6**) analogs were inactive at 100 ppm. 3-(4-Methylpiperazin-1-yl)propyl analog **7** showed activity comparable to that of compound **3**. Replacement of the piperazine ring of compound **3** by a pyrrolidine (**8**), piperidine (**9**) or morpholine (**10**) ring led to reduced activity, while the dialkylamino derivatives (**11** and **12**) did not induce any delay in molting into the 5th instar even at 100 ppm, indicating that a cyclic amine moiety is essential for this activity.

Since compound **3** significantly prolonged the duration of the instar, we determined the ecdysteroid titer in the hemolymph of the larvae (Fig. 2). In the acetone-treated control larvae, the titer dramatically increased 4 days after the 3rd ecdysis, and on day 5, the larvae molted into the 5th instar. However, in the larvae treated with compound **3**, the titer remained at almost the basal level during the first 5 days and reached a peak value on day 7. This pattern of ecdysteroid titers was correlated with prolongation of the duration of the 4th instar. However, it remains to be seen whether or not this delay in ecdysteroid increase was due to the suppression of PTTH release.

Several 2,2-diphenylpropionates, including compound **1**, are known to be mammalian mAChR antagonists and their activities are significantly related to the bond distance between the nitrogen atom and the ester group of the 2,2-diphenylpropionates.[4] On the basis of this structural similarity, compounds **3** and **7** in this article, which are new analogs of 2,2-diphenylpropionates, might be reasonable leads for

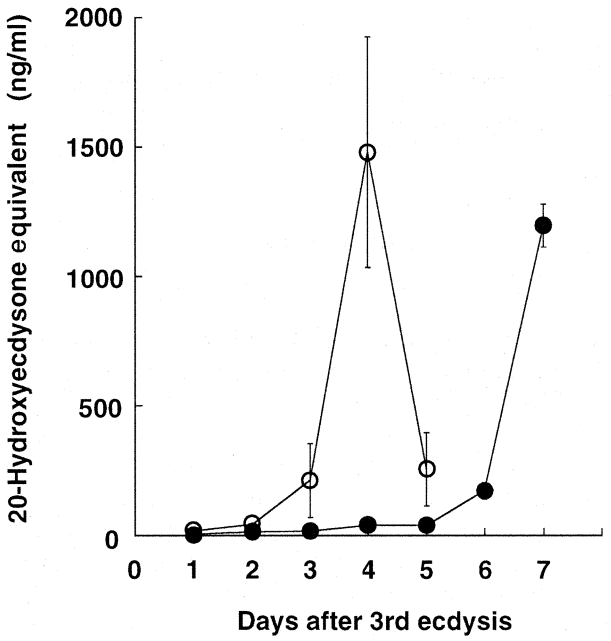


Fig. 2. Change of the Hemolymph Ecdysteroid Titer in 4th Instar Larvae of the Silkworm.

The ecdysteroid titers were determined by an enzyme-linked immunosorbent assay and are expressed as equivalents of 20-hydroxyecdysone as described by Pascual *et al.*[6] The diet containing compound **3** at 50 ppm was administered throughout the 4th larval period. Each point represents the mean from 5 to 20 larvae with S.D. Acetone-treated control (open circles); compound **3** treated (closed circles).

the development of new mAChR antagonists in insects.

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5) Compound **3**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07 (3H, t, J=7.3 Hz), 1.73–1.77 (2H, m), 1.91 (3H, s), 2.21–2.25 (2H, m), 2.37–2.63 (10H, m), 4.18 (2H, t, J=6.4 Hz), 7.21–7.30 (10H, m). *Anal.* Found: C, 63.10; H, 7.59; N, 6.13%. Calcd. for $C_{24}H_{34}O_2N_2$-Cl_2 (HCl salt): C, 63.57; H, 7.56; N, 6.18%.
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