50 μM $CaCl_2$ stimulated aggregation. A higher concentration of $CaCl_2$ seemed to be inhibitory.
Specificity of calcium was tested. Mg^{2+}-EDTA pulses and sustained $MgCl_2$ application had no effect. When EGTA was used instead of EDTA, it induced csA similarly.
This study shows that Ca^{2+}-EDTA pulses stimulated the formation of EDTA resistant cell contacts as well as cAMP pulses did. As in the case of cAMP, pulse application of calcium could induce cell contacts in a shorter period than sustained application. It has been reported that 3 pulses of $CaCl_2$ induced spontaneous oscillation of light scattering in cell suspension. The induction of cell contact by Ca^{2+}-EDTA pulses may be due to triggered cellular oscillations. Calmodulin[9] may take part in this process.

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Antifeedant activity of precocenes and analogs on *Rhodnius prolixus*[1]

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Summary. Precocene and analogs added to the meal of 4th instar larvae of *Rhodnius prolixus* were tested as antifeedants. While precocene II had a strong antifeedant effect (ED_{50} = 48 μg/ml), the other compounds showed no drastic inhibition of feeding (ED_{50} > 140 μg/ml). ATP, a phagostimulant, did not reverse the antifeedant action of precocene II. The mechanism of feeding inhibition is discussed.

Precocenes are plant derived substances that induce precocious metamorphosis in immature hemipterans and prevent ovarian development in some adult insects[3-6]. Precocenes are selectively cytotoxic to the secretory cells of the corpus allatum of the insect, thereby eliminating the production of juvenile hormone[7-12]. However, Slama[13] concluded that premature metamorphosis and ecdysis prevention induced by precocene in certain insects were due to its antifeedant action, as prothetelic insects always appeared among the larvae with the most disturbed growth, close to lethality. In fact, Slama found that precocene profoundly inhibited feeding and growth as well as oviposition in *Pyrrhocoris* and *Dysdercus*. He concluded that the latter effect was identical to that obtained with females treated by the antifeedants myristicin and elemicin. In contrast, although Bowers and Ferugia, and Bowers and Aldrich[14,15] had noted a disinclination by adult female milkweed bugs to feed following treatment with just subtoxic dosages of precocene II, at effective sterilizing concentrations substantial feeding and weight gain occurred. In similar experiments with the milkweed bug it was demonstrated that the main difference in weight between normal and precocene-treated insects was due to the inhibition of ovarian development in the latter[16]. These results emphasize that the antifeedant and anti-hormonal actions of precocene are distinct and especially that the antihormonal activities are not the result of a reduction or elimination of food intake. Considering antifeedants as substances which when tasted or initially consumed can result in cessation of feeding either temporarily or permanently depending on potency, we reported recently that precocene II, added to the diet, acted as a feeding deterrent for *Rhodnius prolixus*, reducing the amount of blood consumed by this insect[17]. We now describe the inhibition of feeding induced by precocene and its analogs in the bloodsucking bug *Rhodnius prolixus*.

Materials and methods. Precocene II, ethoxy precocene (i.e., 7-ethoxy-6-methoxy-2,2-dimethyl chromene[18]) and the chromane of precocene II were synthesized by one of us (W.S.B.); precocene I was purchased from Sigma Chemical Co. All other reagents were of analytical grade. Following ecdysis, the 4th-instar larvae of *R. prolixus* were starved for 20-30 days and then fed on blood or artificial food containing precocene through a special feeding apparatus[19]. Precocenes dissolved in ethanol were added to the citrated blood or artificial diet (1 μl of Tween 80, 20 μl of ethanol plus sample, 10 ml of 30 μM ATP in 0.15 M NaCl) at doses ranging from 10 to 100 μg/ml. Control experiments showed

Table 1. Inhibition of feeding of *Rhodnius prolixus* by the precocenes and analogs

Substances	Effective dose (ED_{50}) (μg/ml)
Chromane of precocene II*	> 300
Precocene I*	260
Precocene II*	48
Precocene II**	43
Ethoxy precocene*	140

* Drugs diluted in blood; ** drugs diluted in artificial diet.

Table 2. Effect of ATP on the inhibition of feeding induced by precocene II in *Rhodnius prolixus*

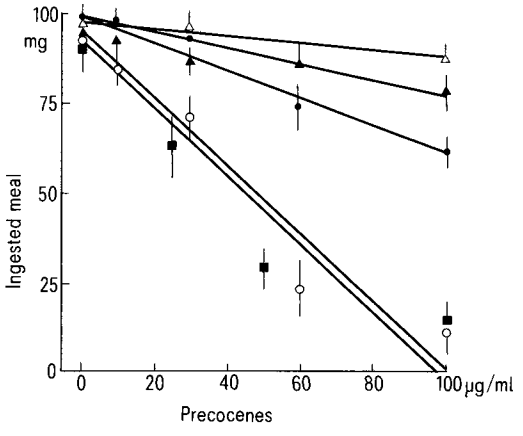
ATP (μm)	Precocene II (μg/ml)*	Meal ingested (mg)
30	-	94.5 ± 5.5
30	50	29.0 ± 6.4
300	50	36.5 ± 7.0
3000	50	34.8 ± 5.6

* Artificial meal.

that ethanol at this concentration did not disturb *Rhodnius* feeding. The insects were allowed to feed for 30 min. The intake of blood was determined by the difference in body weight measured before and after feeding. Tests were performed on groups containing 25–30 insects. The effective antifeedant concentrations (ED_{50}) were determined by computing the linear regression, method of least squares[20], of the ingested meal against the dosage of precocenes.

esults and discussion. The effects of precocene I, II and derivatives on feeding are illustrated in the figure and table 1. As can be seen only precocene II showed a strong antifeedant effect. The other precocenes, as well as the chromane of precocene II, had no drastic antifeedant action at doses up to 100 µg/ml of blood. Table 1 shows that the effective antifeedant doses (ED_{50}) of chromane, precocene I and ethoxy precocene were (> 140 µg/ml) much higher than that of precocene II (48 µg/ml of blood). The figure also shows that the effect of precocene II on the inhibition of feeding was independent of the meal quality; using artificial diet we observed the same ED_{50} as noted for the blood meal (43 µg/ml).

Besides the results illustrated we could also observe that all precocene treatments revealed similar delayed toxicity at doses higher than 30 µg/ml of blood, while the chromane of precocene II had no toxic effect. Since it has been shown that *R.prolixus* is stimulated to feed by ATP[21], we wondered what effect ATP might have on the feeding inhibition induced by precocene II. Table 2 demonstrates that the reduced meal size was an effect of precocene II and was not reversed by addition of ATP to the meal. Our data clearly indicate that change in the chemical structure of precocene II results in great loss of antifeedant activity. The 6,7-dimethoxy group in the 2,2-dimethylchromene (precocene II) seems to be essential for the antifeedant property of precocene. If one of these substituents is replaced by 7-ethoxy (ethoxy precocene) or H- (precocene I) the antifeedant activity is vastly decreased. Although chemical inhibition of feeding has been studied in detail for a few phytophagous insect species, the mechanism(s) of action could function by blocking the input from receptors normally responding to phagostimulants or by stimulating specific 'deterrent' cells[22]. It is known that the former mechanism may be reversed by increasing the amount of phagostimulants[23]. In our case it was not possible to reverse the antifeedant action of precocene by increasing the ATP level (table 2). Alternative explanations related to suspected biochemical interactions of the precocenes are also possible. The precocenes are presumed to undergo biotransformation into reactive epoxides through the action of monooxygenase enzymes within the corpora allata. These reactive intermediates alkylate cellular elements within the allatal cells resulting in cell death and loss of juvenile hormone secretion[12,24]. Many other insect tissues, especially the gut and fat body, contain monooxygenases which may be capable of similar biotransformation of the precocenes[25] The antifeeding response induced in *Rhodnius* following the ingestion of precocene II may be due to a direct cytotoxic action on gut tissues which contain activating monooxygenases. Schooneveld[26] has observed degenerative changes occurring within allatal cells within 90 min of precocene treatment. A similar rapidly induced necrosis of gut tissues could explain the diminution in feeding. Since precocene I, II and ethoxy precocene are all potent antijuvenile hormonal agents the difference in antifeeding activity among them might be due to a specificity of the gut monooxygenases for precocene II. The special feeding behavior of[27] the hematophagous bug *Rhodnius prolixus* makes it an ideal model to investigate this phenomenon and antifeedant substances in general.



Inhibition of feeding by the precocenes and derivatives incorporated into the meal of 4th instar larvae of *Rhodnius prolixus*. △ Chromane of precocene II/ml of blood; ▲ precocene I/ml of blood; ● ethoxy precocene/ml of blood; ■ precocene II/ml of blood; ○ precocene II/ml of artificial diet.

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