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Effects of ingested phytoecdysteroids in the female soft tick *Ornithodoros moubata*

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Abstract. The effects of the ingestion of some phytoecdysteroids were studied in the soft tick *Ornithodoros moubata*. Supernumerary moulting and malformations of first leg pairs were obtained with 22-oxo-20-hydroxyecdysone, 20-hydroxyecdysone-22-acetate, and 20-hydroxyecdysone-22-benzoate. Egg-yield was reduced with 20-hydroxyecdysone-22-acetate and carthamosterone. Finally, drying-out of eggs was observed with carthamosterone and 22-deoxy-20,26-dihydroxyecdysone. In addition, we demonstrated that there is a correlation between the number of completed gonotrophic cycles and the impossibiltiy of inducing supernumerary moulting.
Key words. Tick; *Ornithodoros moubata*; ecdysteroids.

In this paper, we present experiments on the effect of phytoecdysteroids on the reproductive physiology of a tick species. Particularly, in order to determine the importance of the 22-OH position on the efficiency of ecdysteroids as inhibitors of vitellogenesis, we have investigated the effects of phytoecdysteroids modified at the C-22 position when fed to female ticks.

Ecdysteroids are structural analogues of ecdysone, a polyhydroxylated steroid isolated from *Bombyx* pupae, which was the first moulting hormone to be described. Many further ecdysteroids have been isolated or characterized from animals in groups as diverse as arthropods, molluscs, worms, cnidarians and possibly echinoderms[1]. Whereas in arthropods, the role of ecdysteroids as moulting hormones is well established (for chelicerates, crustaceans and myriapods, see refs 2, 3; for insects, see ref. 4), their role in other invertebrates is not yet clear. In addition, ecdysteroids also have roles in reproductive physiology, which are better described for insects[5]. As well as 'zooecdysteroids', more than one hundred ecdysteroids, the 'phytoecdysteroids', have been isolated from plants, in which they are found in much higher concentrations than in arthropods[6]. Many plant families are known to contain phytoecdysteroids, e.g. the Caryophyllaceae[7]. Soon after their discovery, it was suggested that these molecules could protect plants from phytophagous insects or nematodes.

Both ecdysteroids and a family of sesquiterpenic hormones, the juvenile hormones (JH) are involved in the regulation of vitellogenesis in insects[5,8,9]. In the argasid tick *Ornithodoros moubata*, JH-like compounds as well as ecdysteroids seem to control the gonotrophic cycle[10–12]. Topical application of JH induced vitellogenesis in virgin females[13], and small amounts of ingested 20-hydroxyecdysone (20E) induced resorbtion of vitellus in vitellogenic mated females[10]. When females ingested large amounts of ecdysone or 20E, vitellogenesis was also suppressed during an induced supermoulting cycle[14,15]. The same effects were also obtained by using much lower quantities of a synthetic ecdysteroid lacking 22- and 25-OH groups, i.e. 22,25-dideoxy-ecdysone[14]. It is well established that ticks can efficiently inactivate ingested ecdysteroids through conjugation of the 22-OH with long-chain fatty acids[16–18].

Materials and methods

Animals. Engorged and mated females of *Ornithodoros moubata* were bred in the laboratory. They were fed on defibrinated pig blood, through a parafilm membrane. Mating was brought about by keeping the females with an excess of males during 24 hours. Thereafter, they were weighed and placed individually in tubes plugged with cotton, at 27 °C with 60% relative humidity, in the dark. Under these conditions, egg-laying occurred 10 ± 1 days after feeding.

Phytoecdysteroids. The 20-hydroxyecdysone-22-acetate (20E22Ac), isolated from *Silene otites* (Caryophyllaceae), 20-hydroxyecdysone-22-benzoate (20E22Benz), isolated from *Silene scabrifolia*, 22-oxo-20-hydroxyecdysone (22oxo20E), isolated from *Serratula tinctoria* (Asteraceae), 22-deoxy-20, 26-dihydroxyecdysone (22d20,26E), isolated from *Silene nutans*, and carthamosterone (fig. 1), isolated from *Leuzea carthamoides* (Asteraceae), were purified by HPLC from dried plants[7,19,20]. Except for carthamosterone which was weighed on a precision balance (this compound bears an unsaturated lactone absorbing in the UV), the quantity of other ecdysteroids was estimated by UV absorbance of the 7-ene-6-one at 242 nm in ethanolic

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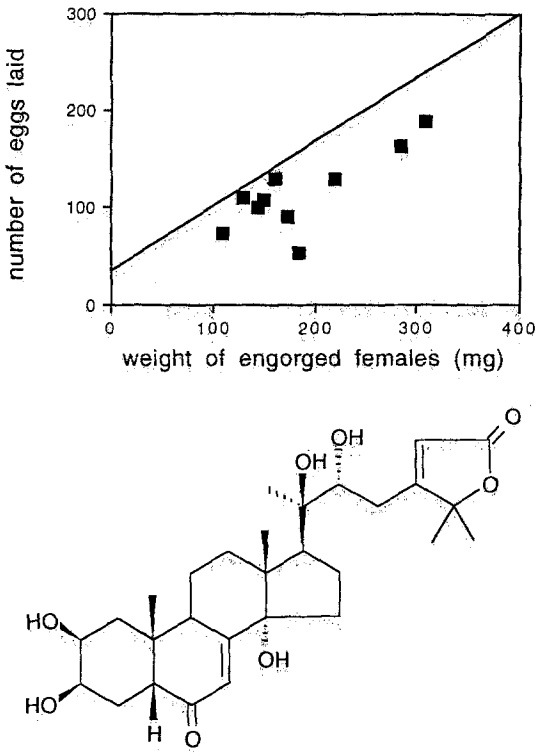

Figure 1. Effects of ingestion of 0.5 µg/ml blood containing carthamosterone on the oviposition. Below, the chemical formula of carthamosterone. Egg-yield of treated females (black squares are individual values) is reduced compared to that of non-treated animals (regression line, equation $y = 24.43 + 0.48 \times R^2 = 0.606$).

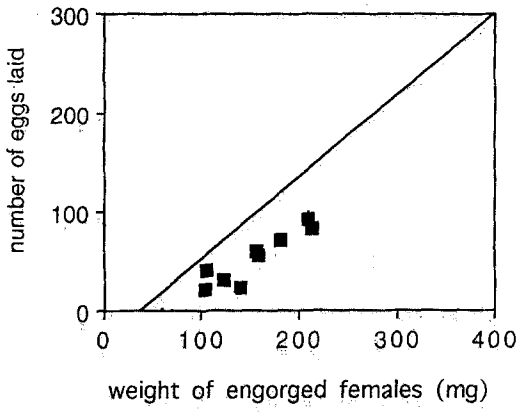

Figure 2. Effects of ingestion of 0.5 µg/ml blood with 20E22Ac on oviposition. Egg-yield of treated females (black squares are individual values) is reduced compared to that of non-treated animals (regression line, equation $y = -36.07 + 0.58 \times R^2 = 0.849$).

solution, and calculation using the value epsilon-m = 12,400.

Ingestion of ecdysteroids. Ecdysteroids were dissolved in 20 µl ethanol and mixed with 3 ml pig blood, which was used to feed 10 *Ornithodoros moubata* females. Because the number of eggs laid is highly correlated with the weight of engorged females 24 hours after feeding[10], we could measure the impact of the treatments on the egg-yield by weighing the treated and control (ethanol fed) females and counting the number of eggs they laid.

Scanning electron microscopy. Specimens were fixed in 70% ethanol, gradually dehydrated with ethanol followed by acetone and then air-dried. They were gold spattered and viewed in a ISI Super Mini SEM.

Results

Effects of different ingested phytoecdysteroids on oviposition. Nine females were fed with 0.5 µg/ml blood containing 22-deoxy-20,26-dihydroxyecdysone (22d20,26E). Seven females oviposited a normal number of eggs 11 ± 1.2 days after feeding. However, all the eggs dried out, suggesting a dysfunction of Géné's organ which secretes the waxes that prevent egg desiccation. Ten other females were fed with blood containing 0.17 µg/ml carthamosterone. All these females laid a reduced number of eggs as compared to controls (fig. 1) 12.6 ± 2 days after feeding. As previously noted for 22d20,26E, the eggs dried out, also suggesting an alteration of Géné's organ. Ten females were fed with 0.5 µg/ml blood with 20E22Benz. Nine of them died 19 days later. At a lower dose (0.05 µg/ml) used with 5 females, none died, and 4 of the 5 females moulted 10 days after feeding. Ten females were fed with 0.5 µg/ml blood containing 22oxo20E. This treatment induced 3 successive moulting cycles without exuviation. When the 3 cuticules were removed, the animals presented legs and mouth parts considerably atrophied (data not shown). Then we fed 13 females with only 0.1 µg/ml 22oxo20E. At this lower dose, 3 females performed 'enclosed moulting', 7 mounlted 17 ± 1.7 days after feeding, and 3 oviposited eggs 13 ± 1.7 days after feeding. Finally, 21 females were fed with blood containing 0.5 µg/ml 20E22Ac. All the females moulted 11.8 ± 1.8 days after feeding. All of them laid eggs 11.8 ± 1.8 days after ecdysis, which corresponds approximately to a normal oviposition time. However, egg-laying was reduced (fig. 2) and legs and mouthparts of the animals were atrophied (fig. 3) as already observed by Connat et al. after ingestion of 22,25-dideoxyecdysone[14].

Influence of the age and of the number of vitellogenic cycles on the induction of a supermoulting cycle. In the previous experiments, all the females used had performed their imaginal moult less than 20 days before the ingestion of ecdysteroids. These animals are called 20-day-old females. After feeding, they were mated for the first time, so they had never laid eggs before the experiment. Such females, when they had ingested 0.5 µg 20E22Ac/ml blood, had always undergone supermoulting cycles before oviposition (fig. 4). In a new series of experiments, we treated older females, which had already laid eggs, with the same dose of 20E22Ac. The six mated 75-day-old females, which had oviposited once, were fed with 20E22Ac. All moulted 16 ± 2.3 days after the blood meal, then only 3 of them laid eggs 9.6 ± 2.3 days after ecdysis. We observed that the pro-

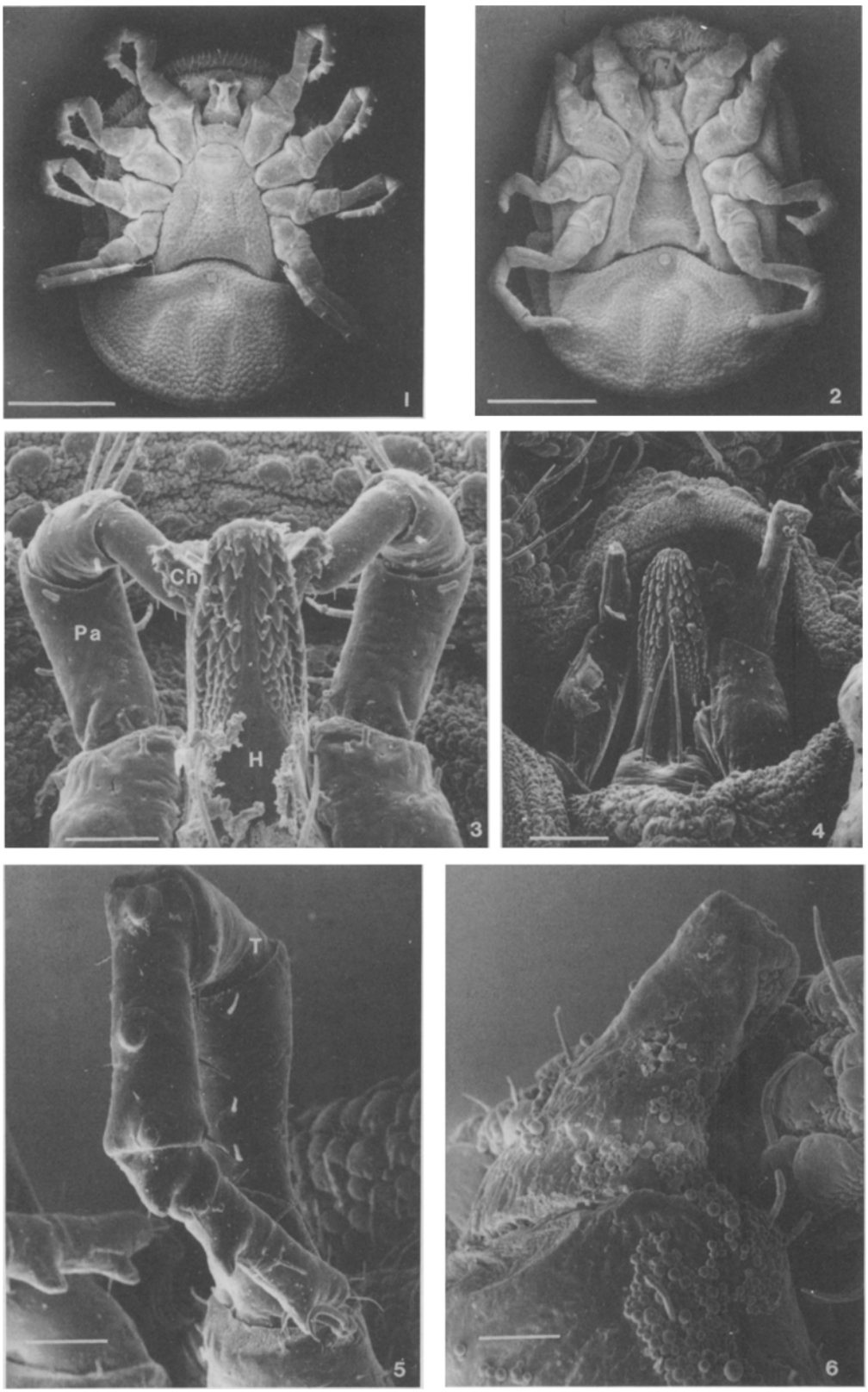

Figure 3. Scanning electron microscope pictures of females which have moulted after ingestion of 0.5 µg/ml blood containing 20E22Ac[2,4,6], compared to non-treated animals[1,3,5]: *1* non-treated female; *2* treated female, the first two pairs of legs are atrophied: all segments are missing except for coxa and the trochanter, and thus the Haller's organ of the first pair of legs is also missing; *3* Capitulum of non-treated female; *4* Capitulum of treated female, the palpae are atrophied; *5* first pair of legs of non-treated female; *6* atrophied leg of treated female (H = Hypostoma, Ch = Che-licerae, Pa = Palpae, T = Tibia). The bar represents 2 mm in pictures *1* and *2*, 100 µm in pictures *3* and *6*, and 200 µm in pictures *4* and *5*.

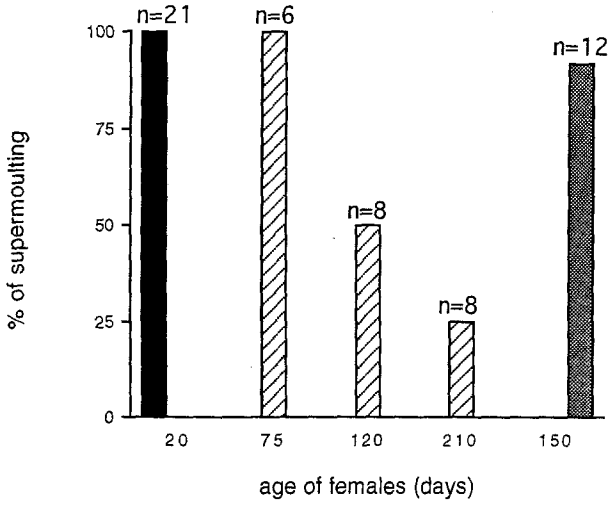

Figure 4. Influence of the age and number of completed gonotrophic cycles in the induction of supermoulting cycles after ingestion of 20E22Ac (0.5 µg/nl blood): mated females (black), mated females which had already laid eggs (hatched), and virgin females (grey).

portion of supermoulting females decreased as the females became older (fig. 4). We treated 8 mated females which had already oviposited once, but which were 120 days old. Only 4 moulted 15 ± 2.5 days after the blood meal. One female oviposited 12 days after being fed while another did not lay eggs within 30 days, and 2 females died after 12 days. Finally, we treated 8 210-day-old mated females, which had already laid eggs twice. In this latter case, only 2 moulted, 4 laid eggs 15.5 ± 4 days after blood meal, and 2 did not lay eggs. In order to find out whether the number of vitellogenic cycles completed or the age of the female was more important in preventing the induction of a supermoulting cycle, we fed twelve 150-day-old virgin females with 0.5 µg 20E22Ac/ml blood. Eleven of them moulted 11.7 ± 2 days after the blood meal (fig. 4).

Discussion

In ticks and most insects, in contrast to many crustaceans and some primitive insects, the adults never moult. However, the moulting of the adult integument of *Ornithodoros moubata* has been observed after the ingestion of 20 µg of ecdysone, 20E, or makisterone A per ml blood[14], 5 µg/ml of cyasterone[11], and 0.035 µg/ml of 22,25-dideoxyecdysone (22,25dE)[14]. The higher activity of 22,25dE could be related to the lack of a 22-OH group; the argasid ticks inactivate ecdysteroids by conjugating them with a long-chain fatty acid in C-22[18]. When the animals were fed with a phytoecdysteroid lacking a 22-OH group, the 22d20,26E, we did not obtain any supermoulting: this could be due to the presence in this molecule of a 26-OH group, which is known in insects to strongly reduce moulting hormone activity[21]. More conclusive experiments would need to be done with, for example, 22-deoxy-20-hydroxyecdysone (also called taxisterone).

The phytoecdysteroids in which 22-OH is replaced by another chemical group were active in *Ornithodoros moubata*. Supermoulting cycles were obtained with both esters (20E22Ac and 20E22Benz) and with 22oxo20E. The latter was probably inactivated at a slower rate, thus inducing the treated animals to perform several successive moulting cycles. When a moulting cycle was just completed, a second was probably initiated by a residual amount of the compound. These effects have also been reported in a closely-related argasid, *Ornithodoros porcinus*[15].

In addition, the physiological state of the females could influence the reaction of the integument to exogeneous ecdysteroids. Our experiments with 20E22Ac showed clearly that supermoulting was more difficult to induce in females which had already completed several gonotrophic cycles, and which were older. Consequently, vitellogenic and artificial moulting processes are not independent of each other, and might even be mutually exclusive. It is suspected that endogenous ecdysteroids could play a role in reproductive physiology, inhibiting vitellogenesis on *Ornithodoros moubata* virgin females[10]. This is consistent with our present experiments, where 20E22Ac significantly reduced the egg-yield. We wonder, however, whether this is a direct or indriect effect of ecdysteroids, because a supernumerary moulting cycle took place before the gonotrophic cycle. This previous moulting cycle could use up some of the energy supplied by the blood meal, which is necessary for complete vitellogenesis. However, after ingestion of carthamosterone, the animals did not moult, whereas the egg-yield was reduced. This indicates that ecdysteroids can have a direct effect on vitellogenesis.

In addition, the supernumerary moults also provoked malformations of the palpae and the first two pairs of legs (ref. 14 and fig. 3). The fact that these malformations were more important in anterior parts could be correlated with the existence of a possible temporal antero-posterior gradient in argasids, comparable to the gradient found during the moulting of nymphal ixodids[22].

The phytoecdysteroids we tested here had drastic effects: inhibiting egg-laying, reducing the egg-yield, and evoking supermoulting with atrophy of mouthparts and anterior legs. Some of these active ecdysteroids are synthesized by plants, where they occur in higher concentrations than are found in arthropods. They can represent up to 3% of the dry weight of the plant, whereas they never exceed 0.025% of the dry weight in terrestrial arthropods[6]. Furthermore, they are active in very low doses in ticks. For example, in *Ornithodoros moubata*, ingestion of 150 ng 20E22Ac provokes supermoulting and reduces egg-laying and 150 ng of 20E22Benz is lethal.

A better understanding of the effects of ecdysteroids on ticks could be very useful in developing ways of preventing infestation of cattle. Knowledge of the pathways of detoxification of ingested phytoecdysteroids could allow the development of more active products. An alternative strategy was recently proposed by Karr et al.[23,24], consisting of the immunization of vertebrates against ecdysteroids. The presence of antiecdysone antibodies in mammalian serum evokes a strongly reduced fecundity in blood-sucking arthropods, which could in turn reduce the ectoparasite population. These possibilities should be fruther investigated.

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