

reverses the anti-reproductive effect of blindness in hamsters, while in the rat anosmia magnifies the otherwise small inhibitory effect of blindness<sup>8</sup>. Regardless of the nature of the blinding-anosmia interaction

which facilitates the response of the rat gonad to the pineal, it is apparent that both the pineal gland and dual sensory deprivation may have independent effects on the reproductive system of young female rats.

- 1 This work was partially supported by state institutional funds and by NSF grant No. PCM 74-06276-276-A02. The radioimmunoassays of the steroids were carried out with the aid of the Radioimmunoassay Core which is supported by USPHS grant No. 1T30 HD-10202. We also thank Constance Stahl for typing assistance.
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### Presence of ecdysone and ecdysterone in the tick *Amblyomma hebraeum* Koch

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**Summary.** Ecdysone and ecdysterone, the moulting hormones of insects and crustaceans, are also present in nymphs of the tick *Amblyomma hebraeum*. They were demonstrated by means of radioimmunoassay and of gas-liquid chromatography combined with mass fragmentography or mass spectrometry of their trimethylsilyl-derivatives.

The occurrence of ecdysteroids as moulting hormones (MH) in insects and crustaceans is now well established<sup>2,3</sup>. In contrast, reports on the chemistry of MH in other groups of the phylum arthropoda are very scarce. The MH of *Limulus polyphemus* (Xiphosura) behaved like ecdysone, ecdysterone and inokosterone in thin layer chromatography (TLC)<sup>4</sup>. In nymphs of the spider *Pisaura mirabilis*, ecdysterone was detected by TLC and radioimmunoassay (RIA)<sup>5</sup>. However, these techniques do not allow precise chemical identification.

The few reports on ecdysteroids in ticks (Acarina, Ixodoidea) deal only with the physiological effects of exogenous hormones. Ecdysteroids ingested with the blood meal induce supermoulting in adults<sup>6,7</sup>. Applied topically, they break larval diapause<sup>8,9</sup>. Injected into the hemocoel, they inhibit oogenesis<sup>10</sup>. We report here the presence of ecdysone and ecdysterone in nymphs of the tick *Amblyomma hebraeum* Koch.

**Materials and methods.** The nymphs were fed on cattle. After dropping from the host, they were kept at 27°C and 70% relative humidity. Moulting to adults occurred about 28 days after dropping. In order to determine the chemical nature of ecdysteroids, 2 samples (62 and 110 g each) of nymphs at day 17 after dropping from the host were homogenized in methanol-water (3:2). After centrifugation, the residue was extracted 3 times with 60% methanol or

methanol. The combined supernatants were purified by precipitation at -18°C followed by centrifugation<sup>11</sup>, and then taken to dryness. The residue was reextracted with methanol. 60 ml of isoamyl-acetate was added and the methanol evaporated. The extracts were submitted to chromatography on a small column of silicic acid which was first eluted with 20 ml of isoamylacetate followed by 20 ml of methylethylketone. The ecdysteroids were eluted with 40 ml of methanol. The eluates were concentrated and subjected to TLC on precoated plates (MERCK, silica gel F<sub>254</sub>, thickness: 0.5 mm). Delipidation was achieved by an initial development in diisopropylether. The ecdysteroids were then separated with chloroform-methanol (7:3). Bands of 1 cm width were scraped off the plates and the substances eluted with methanol and ethylacetate. After

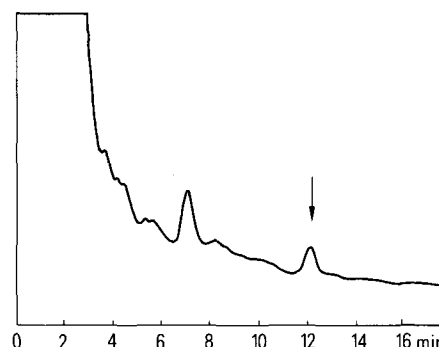


Fig. 1. Chromatogram (total ionization current of the mass spectrometer) of a purified extract of *Amblyomma*. A prominent peak has the same retention time (arrow) as the fully silylated derivative prepared from authentic ecdysterone (Simes, Italy). Unidentified peak at about 7 min retention time does not appear to be an ecdysteroid. Chromatographic conditions: LKB 9000 GLC-MS apparatus; column: 2 m x 2 mm OV.1 (1%) on Gas Chrom P. Temperatures: column 280°C; flash heater and separator 295°C. Flow rate of carrier gas (helium): 30 ml/min.

Relative abundance of characteristic ions detected by MF in a purified extract of *Amblyomma*, at retention time of fully silylated derivative prepared from authentic ecdysone (Simes, Italy). Intensity is given as percent of the main peak, m/e 171, which is characteristic of the side chain. Ions at m/e 567, 582 and 636 are the most abundant ions characterizing ecdysone nucleus and OTMS group on C-22

m/e	171	567	582	636
Biological compound	100	10.7	2.1	6.0
Standard compound	100	13.1	2.2	6.2

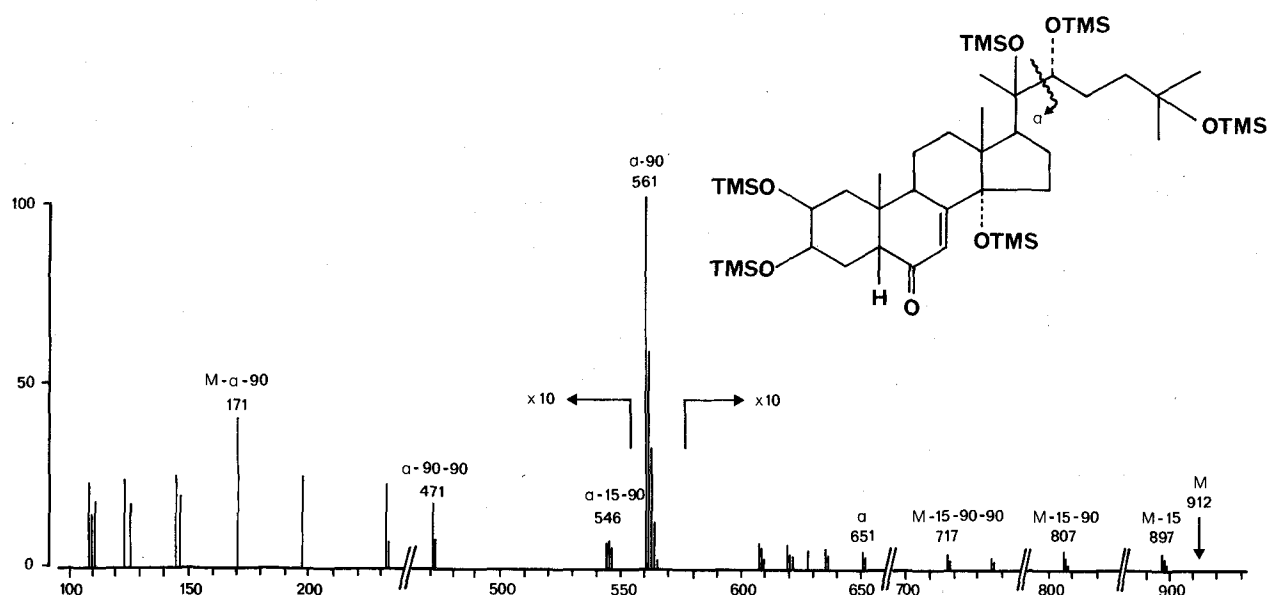


Fig. 2. Mass spectrum of the peak of figure 1 having retention time of hexa-TMS derivative of ecdysterone. This MS is identical with that of the authentic hexa-TMS ecdysterone. The molecular ion ( $M=912$ ) is undetected but is corroborated by peak at  $m/e$  897 ( $M-CH_3$ ) and losses of  $TMSOH$  ( $m/e$  807 and 717). Side chain cleavage ( $m/e$  171 and  $m/e$  651) and strong ion at  $m/e$  561 are characteristic of 20-hydroxyecdysteroids. MS conditions: voltage 3500 V; ionization energy 28 eV; source 310 °C; trap current 60  $\mu$ A.

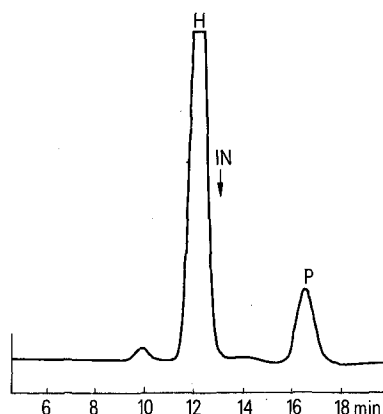


Fig. 3. Mass chromatogram of a purified extract of *Amblyomma* recorded at  $m/e$  561. H and P indicate peaks with same retention times as hexa- and penta-TMS derivatives of ecdysterone respectively. IN indicates retention time of authentic inokosterone-TMS derivative, undetected in this extract.

separation by TLC, the material migrating like standard ecdysone and ecdysterone was silylated with 200  $\mu$ l of trimethylsilylimidazole at 65 °C for 12–15 h. The trimethylsilyl (TMS)-derivatives were extracted 3 times with hexane. The extract was further purified by TLC with toluene-ethylacetate (9:1)<sup>14</sup>. The 3 zones having similar  $R_F$ -values as fully silylated ecdysone and ecdysterone and penta-TMS-ecdysterone were scraped and the material eluted with diethylether. The derivatives were then analysed by gas-liquid-chromatography (GLC) followed by mass fragmentography (MF) or mass spectrometry on an LKB 9000 instrument (for details see figures 1–3). The presence of ecdysteroids in the different extracts was monitored by RIA<sup>11,12</sup>.

**Results and discussion.** A preliminary extraction of about 1500 ticks (= 106 g fresh wt), at day 17 after dropping from

the host, yielded 25  $\mu$ g or more of ecdysteroid-like material as determined by the RIA<sup>11</sup>. Work in progress with another RIA<sup>12</sup> indicates that the level of RIA-positive material fluctuates during the instar and reaches a peak around day 17, when adult cuticle synthesis begins.

After separation by TLC, the RIA<sup>12</sup>-positive material was found to migrate like authentic ecdysterone. For precise chemical identification, the TMS-derivatives of the ecdysteroid-like material were analysed by combined GLC-MF or GLC-mass spectrometry. A prominent peak in the total ionization current was visible having the same retention time as synthetic fully silylated ecdysterone (figure 1). A mass spectrum (MS) was obtained at the peak with the retention time of hexa-TMS derivative (figure 2). It is identical with the MS produced by fully silylated authentic ecdysterone<sup>14,15</sup>.

Ion  $m/e$  561, the most prominent ion of the MS, is characteristic for 20-hydroxyecdysteroids. MF shows (figure 3) that this ion essentially eluted with similar retention time as standard hexa-TMS derivative of ecdysterone. Small amounts of incompletely silylated penta-TMS derivative were also present. Other 20-hydroxyecdysteroids, as inokosterone, have not been detected. From the GLC retention times, from the formation of hexa- and penta-TMS derivatives and from the MS, we conclude that ecdysterone is present in the tick extracts.

We have good evidence for the existence of ecdysone in the ticks. We were not able to get a complete MS because of the small amounts present (about 5% of the amount of ecdysterone). However, the 4 ions detected by MF at  $m/e$  171, 567, 582 and 636, highly characteristic of ecdysone-penta-TMS, do elute at the same time and in the same relative proportions as do the ones from the synthetic derivative of ecdysone (table).

From the chemical data we conclude, therefore, that ecdysone and ecdysterone are present in nymphs of *Amblyomma hebraeum*. This, together with the changing titer of RIA-positive material during the instar and with the physiological effects of exogenous ecdysteroids<sup>6,7</sup>, supports the hypothesis that also in ticks, ecdysteroids are involved in the hormonal control of moulting.

- 1 Acknowledgments. We thank Proff. A. Aeschlimann and C. Noirot and Dr J. Delachambre for their help and encouragement. We are grateful to Proff. Maume and Padieu (Département de Biochimie, Université de Dijon) for the use of the LKB 9000 GLC-MS, and to Drs M.L. De Reggi, M.H. Hirn and Prof. M.A. Delaage for the gift of some RIA-antigens and antibodies. We thank Ciba-Geigy Ltd for the supply with ticks. The work was in part supported by grant Nr. 3.734.76 of the Swiss National Science Foundation and by a grant of the Emil Barrel Stiftung.
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## PRO EXPERIMENTIS

### Supercontraction of conifer chromosomes under the action of alkylating nitrosocompounds

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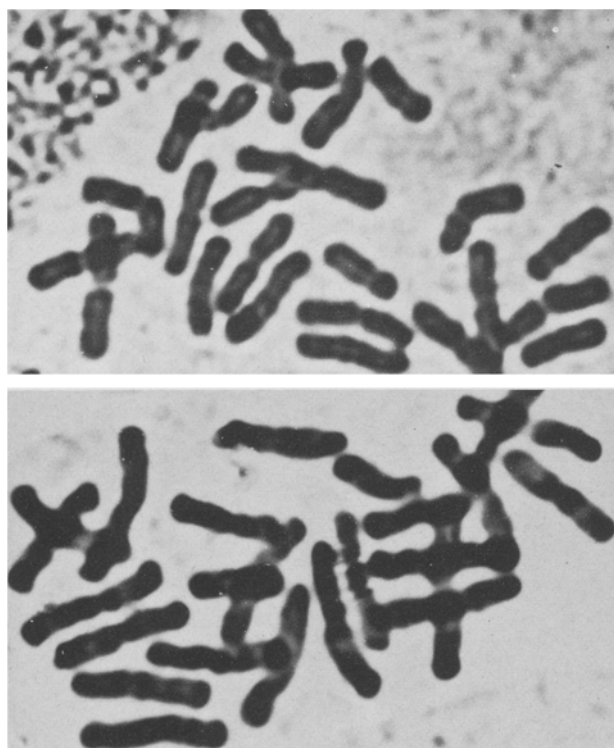
**Summary.** After treatment of seeds of the Norway spruce and of the Scotch pine with alkylating nitrosocompounds, an extremely strong contraction effect on mitotic metaphase chromosomes was observed in the first cell-cycles of the post-treatment period.

The cytogenetic action of chemical mutagens on conifer species has not been sufficiently investigated. However, it is known that the cytogenetic effect of some alkylating nitrosocompounds on the meristematic cells of the Norway spruce, *Picea abies* (L.) Karst.<sup>1</sup>, is similar to their effect on the cells of other higher plants as well as on mammalian cells<sup>2-4</sup>. The main types of chromosome aberrations in-

duced in conifer cells under the action of nitrosocompounds are chromosome fragments of various sizes, lagging chromosomes and bridges (of chromatid and of chromosome type).

When studying the cytogenetic effect of N-methyl-N-nitrosourea and N-methyl-N-nitrosobiuret on the meristematic cells of the Scotch pine (*Pinus sylvestris* L.) and of the Norway spruce, we observed, besides the above-mentioned disturbances, an extremely strong contraction effect on mitotic chromosomes in dividing cells. After treating seeds with small doses (0.001-0.01% aqueous solution at an exposure of 18 h) of these chemicals, the chromosomes in some of the metaphase plates had a typical c-mitotic configuration (figure).

It is known that mitotic chromosomes (in most conifers  $2n=24$ ) are quite long in Gymnosperms. In *Picea* and *Pinus* species the length of the longest chromosomes on mitotic metaphase plates is approximately  $20\text{ }\mu\text{m}$ <sup>5</sup>. It is therefore almost impossible to sort out the mitotic chromosomes on metaphase plates. But under the described experimental conditions, the contraction of chromosomes is such that individualization becomes easy. Contraction was even stronger than in the case of a prolonged colchicine pretreatment (figure). The supercontraction phenomenon of chromosomes is probably connected with a prolongation of cell-cycle phases brought about by the chemicals. At the same time we note a total decline of mitotic activity in the posttreatment period. Supercontraction may thus be a consequence of some general inhibition, rather than of a direct influence on the chromosomes. This response takes place only in the very first cell-cycles after treatment. Later mitoses proceed again normally. Since nuclear division is not synchronous in the embryo, it is possible to observe normal metaphase plates together with metaphase plates having supercontracted chromosomes.



Above: a metaphase chromosome complement of *Picea abies* after N-methyl-N-nitrosourea treatment.  $\times 1900$ .

Below: a metaphase chromosome complement of *Picea abies* after 30-h pretreatment with 0.1% colchicine (same magnification, aceto-orcein squash preparations).

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