Isolation of Ecdysone-3-acetate as a Major Ecdysteroid from the Developing Eggs of the Desert Locust, *Schistocerca gregaria*

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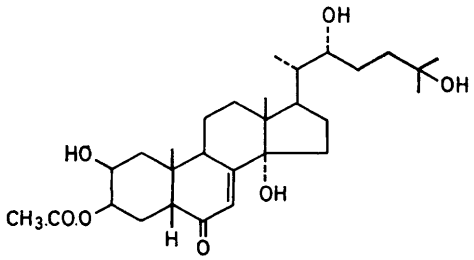
Summary Ecdysone-3-acetate was isolated and characterised from eggs of the desert locust, *Schistocerca gregaria*, at the end of embryogenesis.

ECDYSONE, 2-deoxyecdysone, and 20-hydroxyecdysone have been identified in the newly laid eggs of *Schistocerca gregaria* in the form of highly polar conjugates, which can be hydrolysed with a crude *Helix pomatia* enzyme preparation.[1,2] We have demonstrated that there is appreciable metabolism of ecdysteroids during the last few days of embryogenesis.[3,4] In the present paper we report the isolation from developed eggs of a major and novel ecdysteroid component which has the structure ecdysone-3-acetate (**1**).



(1)

High-polarity ecdysteroids were extracted from eggs[2] (160 g) which were allowed to develop to the end of embryogenesis (day 17). After hydrolysis of the extract with *Helix pomatia* enzymes, the released ecdysteroids were separated by high-performance liquid chromatography (h.p.l.c.) on a reversed phase column (Partisil ODS-3) as described previously.[4] The novel ecdysteroid [300 μg; λ_{max} (MeOH) 242 nm] was isolated† and identified by mass spectrometry and Fourier transform ^{1}H n.m.r. spectroscopy.

The mass spectra included the following major ions: chemical ionisation (NH_3) *m/e*, 524 ($[M + NH_4]^+$, 0·6%), 506 ($[M + NH_4 - H_2O]^+$, 0·3%), 489 ($[M + 1 - H_2O]^+$, 10%), 488 ($[M + NH_4 - 2H_2O]^+$, 10%), 447 ($[M + 1 - 60]^+$, 2%), 446 ($[M + NH_4 - 60]^+$, 2%), 429 ($[M + 1 - H_2O - 60]^+$, 12%), and 99 (100%); electron impact *m/e* 470 ($[M - 2H_2O]^+$, 2%), 428 ($[M - H_2O - 60]^+$, 1%), 410 ($[M - 2H_2O - 60]^+$, 2%), 395 ($[M - 2H_2O - CH_3 - 60]^+$, 1%), 390 ($[M - (C22—C27)]^+$, 3%), 372 ($[390 - H_2O]^+$, 8%), 342 ($[M - (C20—C27)]^+$, 10%), and 99 (100%).

These data suggested that compound (**1**) was an ecdysone acetate. The failure to form a 2,3-acetonide under conditions[5] favourable to the formation of ecdysone 2,3-acetonide indicated that the acetylated hydroxy-group was at either C-2 or C-3. The ^{1}H n.m.r. spectrum (400 MHz; $CDCl_3$) [δ 0·71 (3H, s, 18-Me), 0·97 (3H, d, *J* 7 Hz, 21-Me), 1·03 (3H, s, 19-Me), 1·27 (6H, s, 26/27-Me), 2·14(3H, s, 3-OAc), 4·05 (1H, m, 2-H, $w_{\frac{1}{2}}$ 20 Hz), 5·23 (1H, m, 3-H, $w_{\frac{1}{2}}$ 7 Hz), and 5·87 (1H, d, 7-H)] was consistent with the compound being ecdysone-3-acetate.[5]

The ester was hydrolysed by treating it with a 0·6% solution of K_2CO_3 in methanol–water (9 : 1, v/v). The ecdysteroid released was recovered in high yield (80%) and was shown to be ecdysone by co-chromatography on h.p.l.c.[6] with authentic material and by mass spectrometry and Fourier transform ^{1}H n.m.r. spectroscopy.

A mixture of ecdysone-2-acetate and ecdysone-3-acetate was prepared and separated from any ecdysone-22-acetate by t.l.c.[5] Ecdysone-3-acetate was separated from ecdysone-2-acetate by h.p.l.c. on a reversed phase column (Partisil ODS-3).† The material was stored at −20 °C without solvent, since under these conditions migration of the acetate group from C-3 to C-2 was kept to a minimum. Migration of acetate groups between vicinal hydroxy-groups is favoured in aqueous acidic solution[7] and has been reported during derivatisation reactions of ecdysteroids.[8] The compound isolated from day-17 eggs co-chromatographed with the synthesised ecdysone-3-acetate on h.p.l.c. (Partisil ODS-3 and APS-Hypersil),[6] and both the mass and Fourier transform ^{1}H n.m.r. spectra of the two compounds were indistinguishable.

On silica gel t.l.c.,‡ ecdysone-3-acetate could not be separated from ecdysone-2-acetate and 3-dehydroecdysone. The latter compound has been reported to be a metabolite of ecdysone in some insect species (see ref. 9 and literature cited therein). Therefore, it is important that future characterisations of ecdysone-3-acetate or 3-dehydroecdysone be confirmed by co-chromatography on both adsorption and reversed phase systems.

On emergence of the embryos, ecdysone-3-acetate was the major component of the insect's polar ecdysteroid conjugate fraction. Acetylation and conjugation of ecdysone with polar moieties would protect the first instar larva from exposure to harmful levels of moulting hormone of maternal origin.[2]

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† Retention volumes on a Whatman Magnum 9 Partisil ODS-3 column (50 cm × 9.4 mm i.d.) eluted with a linear gradient (40 min) of 40% → 80% methanol–water were: 20-hydroxyecdysone, 82 ml; ecdysone, 110 ml; ecdysone-3-acetate (**1**), 128 ml; ecdysone-2-acetate, 145 ml.

‡ R_f values on silica gel t.l.c. (solvent, chloroform–methanol, 4 : 1) were ecdysone-3-acetate (**1**), ecdysone-2-acetate, and 3-dehydroecdysone, 0·62; ecdysone, 0·37; 20-hydroxyecdysone, 0·30.

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