Isolation of 2-Deoxy-20-hydroxyecdysone and 3-Epi-2-deoxyecdysone from Eggs of the Desert Locust, *Schistocerca gregaria*, during Embryogenesis

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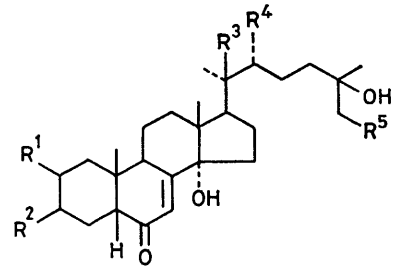
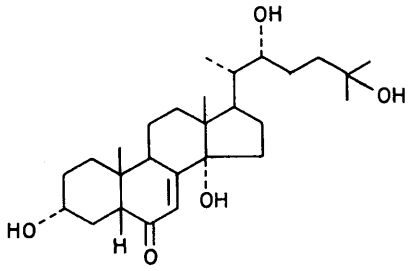
Summary 2-Deoxy-20-hydroxyecdysone and 3-epi-2-deoxyecdysone have been isolated from developing eggs of the desert locust, *Schistocerca gregaria,* and their structures determined.

THE ovaries of several species of insects have been shown to synthesise ecdysteroids (insect moulting hormones), which in most cases are passed largely into the eggs.[1] In the desert locust, *Schistocerca gregaria,* the ecdysteroids which have been identified[2,3] in newly laid eggs include ecdysone (**1**), 2-deoxyecdysone (**2**), and 20-hydroxyecdysone (**3**), together with probable traces of 26-hydroxyecdysone (**4**), all being present primarily as polar conjugates hydrolysable with a crude *Helix pomatia* enzyme preparation. In this

insect, ecdysteroids are also present primarily as conjugates throughout embryogenesis,[4] which generally lasts 17·5 days at 30 °C. The isolation and identification of 2-deoxy-20-hydroxyecdysone (**5**) and 3-epi-2-deoxyecdysone (**6**) from 17-day-old eggs of *S. gregaria* are now reported; (**6**) has not previously been isolated from insects.



(1) $R^1=R^2=R^4=OH$, $R^3=R^5=H$
(2) $R^2=R^4=OH$, $R^1=R^3=R^5=H$
(3) $R^1=R^2=R^3=R^4=OH$, $R^5=H$
(4) $R^1=R^2=R^4=R^5=OH$, $R^3=H$
(5) $R^2=R^3=R^4=OH$, $R^1=R^5=H$



(6)

Day-17 developing eggs (160 g) of *S. gregaria* were extracted and fractionated as previously described.[3] Ecdysteroids were released from the conjugate fraction by hydrolysis with *Helix pomatia* enzyme and fractionated directly by h.p.l.c. on a reversed-phase column (Partisil ODS-3).† Two u.v.-absorbing compounds [λ_{max} (in MeOH) 242 nm] were isolated [(**5**), 100 μg; (**6**), 125 μg] in addition to the ecdysteroids (**1**)—(**4**) previously identified in newly laid eggs.

The mass spectra of (**5**) included the following ions: chemical ionisation (c.i.) (isobutane) *m/e*, 465 ($[M + H]^+$, 6%); electron impact (e.i.) *m/e*, 428 ($[M-2H_2O]^+$, 0·5%), 392 ($[M-4H_2O]^+$, 2%), 347 {$[M-(C\text{-}22—C\text{-}27)]^+$, 9%}, 284 {$[M-(C\text{-}20—C\text{-}27)-H_2O]^+$, 6%}, 99 {$[(C\text{-}22—C\text{-}27)-H_2O]^+$, 17%}, 81 ($[99-H_2O]^+$, 34%), and 43 (100%). The ^{1}H n.m.r. spectrum (Fourier Transform, 400 MHz, C_5D_5N) of (**5**) showed signals at δ 1·08 (3H, s, 19-Me), 1·27 (3H, s, 18-Me), 1·40 (6H, s, 26/27-Me), and 1·64 (3H, s, 21-Me). Acetylation of (**5**) for 17 h at room temperature gave primarily a diacetate: c.i.–m.s. (NH_3), *m/e*, 513 ($[M + H-2H_2O]^+$, 0·1%), 495 ($[M + H-3H_2O]^+$, 0·5%), 393 ($[513-2 \times 60]^+$, 2%), and 43 (100%).

The mass spectral data suggest that (**5**) is a pentahydroxylated ecdysteroid and are consistent with the presence of hydroxy-groups at C-20, C-22, and C-25. The methyl signals in the ^{1}H n.m.r. spectrum of (**5**) were similar to those of 20-hydroxyecdysone and indicate the presence of hydroxy-groups at C-14, C-20, and C-25.[5] From biogenetic considerations, the final hydroxy-group can probably be placed at C-3. These data taken together indicate that (**5**) is 2-deoxy-20-hydroxyecdysone. This was confirmed by comparison of (**5**) by t.l.c., h.p.l.c. (Partisil ODS-3 and APS-Hypersil)[6] and e.i.–m.s. with an authentic sample supplied by Dr. D. H. S. Horn.

The mass spectra of (**6**) included the following ions: c.i. (isobutane) *m/e*, 449 ($[M + H]^+$, 1%; e.i., *m/e*, 430 ($[M-H_2O]^+$, 4%), 394 ($[M-3H_2O]^+$, 4%), 343 ($[M-5H_2O-Me]^+$, 5%), 332 {$[M-(C\text{-}22—C\text{-}27)]^+$, 8%}, 284 {$[M-(C\text{-}20—C\text{-}27)-H_2O]^+$, 18%}, 99 {$[(C\text{-}22—C\text{-}27)-H_2O]^+$, 100%}, and 81 ($[99-H_2O]^+$, 68%); ^{1}H n.m.r. (Fourier Transform, 400 MHz, C_5D_5N) δ 0·75 (3H, s, 18-Me), 1·01 (3H, s, 19-Me), 1·32 (3H, d, *J* 6 Hz, 21-Me), and 1·41 (6H, s, 26/27-Me).

The c.i. and e.i. spectra of (**6**) were almost indistinguishable from those of 2-deoxyecdysone. The methyl signals in the ^{1}H n.m.r. spectrum of (**6**) were similar to those of ecdysone (**1**)[5] except for the resonance of the C-19 methyl group. The chemical shift (δ 1·01, pyridine) of the C-19 methyl in (**6**) occurs at higher field than that of 2-deoxy-20-hydroxyecdysone (δ 1·08) and 2-deoxyecdysone (**2**).[3] Such an upfield shift has been reported in 3-epi-2-deoxy-20-hydroxyecdysone as compared to 2-deoxy-20-hydroxyecdysone,[7] but is not observed in 3-epi-ecdysteroids possessing a hydroxy-group at C-2.[8]

Acetylation of (**6**) for 17 h at room temperature gave primarily a diacetate: c.i.–m.s. (NH_3), *m/e*, 533 ($[M + H]^+$, 1%); ^{1}H n.m.r. (Fourier Transform, 400 MHz, $CDCl_3$) δ 2·02 (3H, s, 3-OAc), 2·07 (3H, s, 22-OAc),[5] 4·73 (1H, m, 3-H, $w_{1/2}$ 22 Hz), 5·87 (1H, d, 7-H), and 4·90 (1H, m, 22-H, $w_{1/2}$ 16 z). The broad resonance of the axial C-3 proton is in agreement with that reported for 3-epi-2-deoxy-20-hydroxyecdysone.[7]

These data taken together establish the structure of (**6**) as 3-epi-2-deoxyecdysone. This was corroborated by comparison of (**6**) by t.l.c., h.p.l.c. (Partisil ODS-3) and e.i.–m.s. with a sample of 3-epi-2-deoxyecdysone recently isolated from the fern, *Blechnum vulcanicum*.[9]

2-Deoxy-20-hydroxyecdysone is present throughout embryogenesis in *S. gregaria* eggs and is, at least primarily, of maternal origin. This ecdysteroid may be formed from 2-deoxyecdysone. The possibility exists that 20-hydroxyecdysone may arise not only by C-20 hydroxylation of ecdysone but also by hydroxylation of 2-deoxy-20-hydroxyecdysone at C-2. 2-Deoxy-20-hydroxyecdysone has been isolated previously from insects and crayfish.[10] 3-Epi-2-deoxyecdysone is detectable only in developing *S. gregaria* eggs towards the end of embryogenesis (day 14, onwards). This ecdysteroid has not hitherto been reported in insects.

† 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) 40 → 80% methanol–water were: ecdysone (**1**), 110; 2-deoxy-20-hydroxyecdysone (**5**) 120; 3-epi-2-deoxyecdysone (**6**) 154 ml. R_f values on silica gel t.l.c. (solvent, $CHCl_3$–MeOH 4 : 1) in comparison with other known ecdysteroids were: (**6**) 0·60; 2-deoxyecdysone (**2**) 0·56; (**5**) 0·51; ecdysone (**1**) 0·39.

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