Isolation of a New Ecdysteroid, 2,22-Dideoxy-20-hydroxyecdysone, from the Ovaries of the Silkworm *Bombyx mori*

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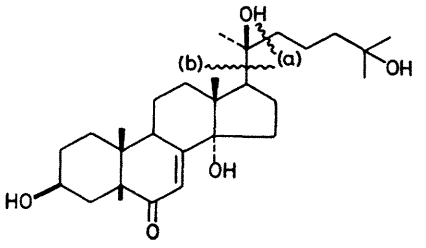
Summary A new ecdysteroid was isolated from the ovaries of the silkworm, *Bombyx mori*, and the structure was determined by mass and n.m.r. spectroscopy to be 2,22-dideoxy-20-hydroxyecdysone.

IN a previous paper,[1] we reported the presence of a new ecdysteroid in the ovaries and eggs of the silkworm, *Bombyx mori*, in addition to 2-deoxyecdysone and ecdysone. We have now determined the structure of this ecdysteroid to be 2,22-dideoxy-20-hydroxyecdysone (**1**).

A mixture of ecdysteroids was isolated from 4 kg of ovaries (from 6000 pharate adults) by the same procedure as reported.[1] After hydrolysing the conjugate fraction with snail juice, the ecdysteroid fraction was obtained by

silicic acid chromatography, and was subsequently separated by preparative t.l.c.† The final purification was carried out by h.p.l.c. using Wakogel ODS and Zorbax-SIL.‡ *Ca.* 1 mg of the new ecdysteroid [u.v.: λ_{max} (EtOH) 245 nm (ϵ 12,000)] was isolated.



(1)

The mass spectrum exhibited important peaks[2] at *m/e* 448 (M^+), 430 ($M^+ - 18$), 415 ($M^+ - 18 - 15$), 412 ($M^+ - 18 \times 2$), 397 ($M^+ - 18 \times 2 - 15$), 394 ($M^+ - 18 \times 3$), 379 ($M^+ - 18 \times 3 - 15$), and 361 ($M^+ - 18 \times 4 - 15$), all of which are associated with deoxyecdysone. Strong fragment peaks at *m/e* 347, 329, and 311 are consistent with the fragments of side chain cleavage at C(20)–C(22) [cleavage (a)] and *m/e* 304, 286, 271, and 253 consistent with the cleavage (b) at C(17)–C(20). The above peaks and strong peaks at *m/e* 234 indicated a 2-deoxyecdysteroid structure having a hydroxy group at the C-20 position. The presence of intense peaks at *m/e* 145, 127, 109, 59, and 41 also indicated a 20,25-dihydroxy side chain. High-resolution electron impact-mass spectrometry (e.i.-m.s.) showed the molecular ion for $C_{27}H_{44}O_5$ at *m/e* 448·3198. Acetylation of the ecdysteroid produced a monoacetate: *m/e* 490 (M^+), 472, 454, 430 by field desorption-mass spectrometry, and *m/e* 472 ($M^+ - 18$), 457, 454, 439, 421, 389, 379, 371, 346, 311, 276, and 216 by e.i.-m.s. These data revealed a 20,25-diol structure rather than the usual 22,25-dihydroxy side chain. The complete structure was elucidated to be 2,22-dideoxy-20-hydroxyecdysone (3β, 14α,20*S*,25-tetrahydroxy-5β-cholest-7-en-6-one) (**1**) from its n.m.r. spectra (200 MHz, $CDCl_3$) [δ 0·86 (3H, s, 18-Me), 1·00 (3H, s, 19-Me), 1·24 (3H, s, 21-Me), and 1·26 (6H, s, 26,27-Me); monoacetate, δ 0·87 (3H, s, 18-Me), 1·01 (3H, s, 19-Me), 1·25 (3H, s, 21-Me), 1·26 (6H, s, 26,27-Me), 2·08 (3H, s, Ac), 5·14 (1H, m, 3α-H), and 5·90 (1H, brs, 7-H)] by comparison with the spectra of 22-deoxy-20-hydroxyecdysone,[3] 2-deoxy-3-*epi*-20-hydroxyecdysone,[4] and 3β,14α-dihydroxy-5β-cholest-7-en-6-one.[5]

The ecdysteroid accumulates in the ovary of the pharate adult of the silkworm in free as well as conjugated forms, together with 2-deoxyecdysone, ecdysone, and 2-deoxy-20-hydroxyecdysone.[6] After egg-laying, the ecdysteroid in eggs decreases to quite a low level. In a bioassay using isolated abdomens of the fleshfly, *Sarvophaga peregrina*, this compound exhibited about one tenth of the activity of ecdysone. The hydroxy group at the C-22 position must affect the biological activity, since the biological activity, as tested by isolated abdomens of *Calliphora stygia*, of 2-deoxyecdysone and 2-deoxy-20-hydroxyecdysone has been reported to be comparable to or slightly greater than that of ecdysone.[7]

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† R_f Values on Merck Kieselgel 60 F_{254} with a solvent system of $CHCl_3$–96% EtOH (4 : 1) of 2-deoxyecdysone, the new ecdysteroid, and ecdysone were 0·54, 0·46, and 0·32, respectively. These correspond to the R_f values of 0·45, 0·35, and 0·27 in our previous paper.[1]

‡ Retention times of 2-deoxyecdysone, the new ecdysteroid, and ecdysone on Zorbax SIL, 15 cm × 4·6 mm internal diameter (i.d.), with CH_2Cl_2–MeOH (7%) were 8·5, 9·2, and 14·6 min, and on Wakogel ODS-10K, 50 × 4·0 mm i.d., with MeOH–H_2O (3 : 2), 19·1, 21·0, and 9·9 min, respectively.

[1] E. Ohnishi, T. Mizuno, F. Chatani, N. Ikekawa, and S. Sakurai, *Science*, 1977, **197**, 66.
[2] K. Nakanishi, in 'Chemistry of Natural Products,' ed. M. N. Kolosov, Butterworth, London, 1971, vol. 7, p. 167.
[3] J. N. Kaplanis, M. J. Thompson, S. R. Dutky, W. E. Robbins, and E. L. Lindquist, *Steroids*, 1972, **20**, 105.
[4] M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Aust. J. Chem.*, 1969, **22**, 1059.
[5] M. N. Galbraith, D. H. S. Horn, and E. J. Middleton, *Aust. J. Chem.*, 1974, **27**, 1087.
[6] E. Ohnishi, T. Mizuno, N. Ikekawa, and T. Ikeda, manuscript in preparation.
[7] Y. K. Yong, M. N. Galbraith, and D. H. S. Horn, *J. Chem. Soc., Chem Commun.*, 1970, 1217.