Identity of the Moulting Hormones of Insects and Crustaceans

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CRUSTECDYSONE (I) a crustacean moulting hormone[1] is now reported as the major moulting hormone of an insect. Fractionation of the extract of the pupae (31 kg.)[2] of the Saturniid oak-silk moth *Antherea pernyi* afforded an active substance (200 μg.), which has the same R_f-value in thin-layer chromatograms and gives the same mass spectrum as crustecdysone isolated from crayfish. As the partition coefficient of crustecdysone in the solvent system consisting of cyclohexane, butanol, and water (6 : 4 : 10) is approximately the same as that reported for β-ecdysone isolated from pupae of the silk moth *Bombyx mori*,[3] there is now little doubt that crustecdysone and β-ecdysone are the same substance. Fractionation of a less polar portion of the extract of 1 ton of crayfish afforded a second highly-active moulting hormone, but in much smaller amount (*ca.* 50 μg.) than crustecdysone (2 mg.).[1] This hormone has an R_f-value of 0·36 (*cf.* 0·16 for crustecdysone) in thin-layer chromatograms using unactivated silica gel (Merck type H) with chloroform–96% ethanol (80–20) as solvent and appears like crustecdysone as an olive green spot with the vanillin–sulphuric acid spray reagent[4] or as a dark spot on silica gel (Eastman Chromatograph Sheet Type K301R) under u.v. light (2540 Å). Too little material was obtained to permit complete purification and further characterization but the R_f-values in partition and adsorption chromatograms were the same as those of ecdysone (II), obtained in small amount from the less polar fractions of the extract of *Antherea* pupae.

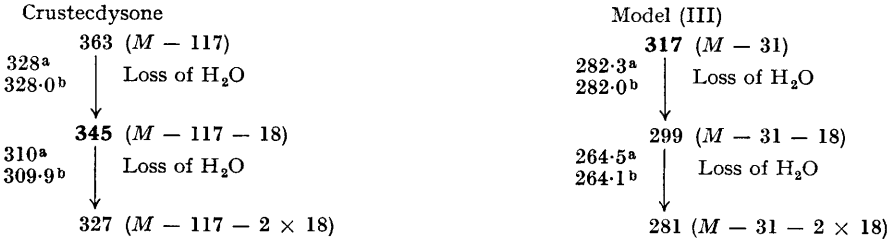
Further support for the structure suggested for crustecdysone (I) has been obtained by a comparison of the n.m.r. and mass spectra of the model compounds (III)[5] and (IV)[6] which contain some of the features of the crustecdysone (I) and ecdysone (II) structures respectively and differ only at the C-20 position. In Table 1 it is seen that the differences ($\Delta\delta$) between the chemical shifts (δ) of the corresponding methyl resonances of the two models and of the two hormones are of the same order. None of the values calculated from published data[7] for the C-18 and C-19 methyl resonances of ecdysone with an additional nuclear hydroxyl group at many other possible positions showed a convincing fit with those observed for crustecdysone. In the mass spectrum of (III) the base peak at m/e $M-31$ corresponds, as does a major peak in the spectrum of crustecdysone (m/e $M-117$) to the oxonium ion formed by simple fission of the C-20–C-22 bond. Metastable peaks indicate that in each case (Table 2) these

TABLE 1. *Chemical shift differences of methyl resonances*

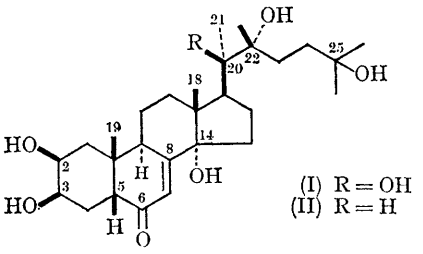
	δ C-18	Δδ C-18	δ C-19	Δδ C-19	δ C-21	Δδ C-21
Model (IV)	0·72		1·07		1·31	
		+0·39		−0·02		+0·19
Model (III)	1·11		1·05		1·50	
Ecdysone	0·74		1·07		1·28	
		+0·46		0·00		+0·28
Crustecdysone	1·20		1·07		1·56	

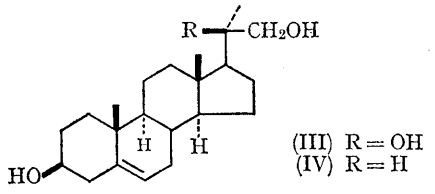
Spectra were measured with pyridine or deuteropyridine solutions.

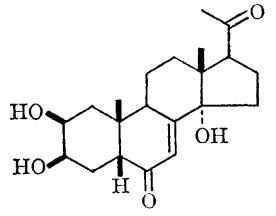
TABLE 2. *Fragmentation pattern of the nuclear oxonium ions* $[R(CH_3)\cdot C{=}\overset{+}{O}H]$ *formed by* C-20–C-22 *bond fission*



Base peaks in bold type. Observed[a] and calculated[b] metastable peaks at left of arrows.







fragment ions then undergo successive loss of two molecules of water. The base peak at *m/e* 31 in the mass spectrum of (IV) indicates that C-20–C-22 bond fission takes place. In this case the side-chain fragment carries all the charge, since no peak at $M-30$, expected from the pattern of fragmentation of ecdysone,[8] was found. In the spectrum of ecdysone, ions due to side-chain fragments also predominate (*m/e* 99 and 81). Both the spectra of (IV) and ecdysone[8] show the presence of abundant $M-15$ or $M-18-15$ species, but peaks due to such ions are not prominent in the spectra of crustecdysone or (III). The closely parallel fragmentation patterns of (I) and (III) on the one hand and (II) and (IV) on the other, and the marked differences between the two pairs of compounds provide convincing support for the structure suggested for (I).

It is likely that ecdysone is the precursor of crustecdysone. The biological hydroxylation of the cholesterol side chain is known to follow a similar route[9] and, as the replacement of the 20α-hydrogen of cholesterol occurs with retention of configuration, crustecdysone can be expected to be 20α-hydroxy-ecdysone (I). Since the biological hydroxylation of cholesterol at C-20 and C-22 leads by C-20–C-22 bond scission to pregnenolone[9] it is possible that crustecdysone is degraded in the same way to the analogous compound (V). Although ecdysone is about twice as active as crustecdysone,[1,3] the latter is much more abundant in extracts of both *Antherea* pupae and crayfish

and may be the hormone chiefly responsible for moulting. However, the relative proportions of the two hormones may vary at critical periods and each in turn may effect different stages of the moulting process.

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