Structure of Crustecdysone, a Crustacean Moulting Hormone

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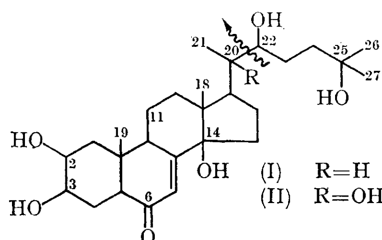
RECENT publications[1,2] on the structure of ecdysone (I), a moulting hormone of insects, have prompted us to report on the structure of a similar hormone,[3] isolated from the sea-water crayfish, *Jasus lalandei*. We propose the name crustecdysone (II) (crustacean ecdysis) for this hormone.

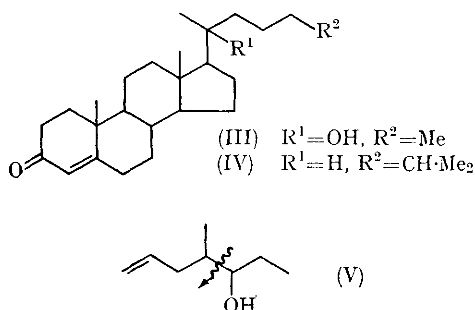


Since crustacean extracts are active in the insect bioassay[4] for ecdysone, the crustacean moulting hormone is considered to be similar to,[5] but not identical with, ecdysone.[6] The isolation of crustecdysone is more difficult[5] than ecdysone and an attempt to isolate the hormone from 3 tons of shrimps, *Crangon vulgaris*, using the ecdysone isolation procedure apparently failed to provide sufficient pure hormone for characterization.[7] We have found that crude extracts of crayfish, at an intermoult stage, have less than 1/100th the activity of comparable extracts of silkworm pupae, and that the ecdysone purification procedure is unsatisfactory for the isolation of crustecydsone, chiefly because the hormone is extensively decomposed when chromatographed on alumina. By means of a long series of purification steps,[8] and using the modified[9] *Calliphora* bioassay of Fraenkel[4] to locate the active fractions, we have isolated the substantially pure, but non-crystalline hormone (2 mg. from 1 ton of frozen crayfish waste, consisting chiefly of ventral thoraxes). The material is highly active in the *Calliphora* test (*ca.* 30,000 *Calliphora* units per mg.) but less active than ecdysone (100,000 *Calliphora* units per mg.).

Crustecdysone has an R_f-value of 0·50 in thin-layer chromatograms using unactivated silica gel (Merck type H) with chloroform–96%-ethanol (60–40) as solvent, and appears as an olive-green spot with the vanillin–sulphuric acid spray reagent[10] or as a dark spot on silica gel (Eastman Chromatogram Sheet Type K301R) under u.v. light (2540 Å). Crustecdysone is more polar in solvent partition systems than ecdysone and shows a stronger hydroxyl absorption in its i.r. spectrum at 3500 cm.$^{-1}$ From its mass spectrum (parent ion m/e 480) crustecdysone contains one hydroxyl group more than ecdysone. The i.r. absorption (1655 cm.$^{-1}$ in KBr) and the u.v. absorption (λ_{max} 242 mμ, ϵ *ca.* 8,000 in ethanol) indicate the presence of an $\alpha\beta$-unsaturated ketone chromophore, presumably the same 7-en-6-one chromophore as in ecdysone (I). The u.v. absorption maximum of crustecdysone measured in ethanolic 1N-hydrochloric acid showed a slow shift to longer wavelength at room temperature and on heating to 70° for 15 minutes two new maxima appeared at 248 and 295 mμ. Thin-layer chromatography showed the reaction product to be a mixture of two substances less polar than crustecdysone. Since ecdysone undergoes a similar change in the presence of acid, due to the elimination of the 14-hydroxyl group and formation of two isomeric dienones,[2] it is concluded that crustecdysone also has a 14-hydroxyl group.

The n.m.r. spectra of ecdysone and crustecdysone in deuteropyridine are similar. Both have their strongest peaks at δ 1·38 [attributed to the methyl protons of $-C(OH)(CH_3)_2$], peaks at δ 1·07 (attributed to the C-19 methyl protons) and peaks at δ 6·18 (attributed to the 7-protons). However, the spectra differ markedly in the positions of the C-18 and C-21 methyl signals. The absence of a singlet at δ 0·74 (attributed in the spectrum of ecdysone to the C-18 methyl) and the presence of a signal at δ 1·20 suggests that the crustecdysone structure has a hydroxyl group at a position such that it produces a strong downfield shift in the signal of the C-18 methyl. Also the absence of the doublet at δ 1·28 (attributed in the spectrum of ecdysone to the C-21 methyl) and the presence of a singlet further downfield at δ 1·56 suggests that the hydroxyl group may be located on C-20. This assignment is supported by the fact that in the spectrum of the model compound (III), the signal due to the C-21 methyl appears at δ 1·45 and that the signal of the C-18 methyl appears at δ 1·09 which is 0·41 p.p.m. further downfield than in

compound (IV).[2] The signal of the C-18 methyl of crustecdysone can therefore be expected to appear about 0·41 p.p.m. further downfield than that of ecdysone (*i.e.*, at δ 1·15; *cf.* δ 1·20 observed) if crustecdysone differs from ecdysone only in having a 20-hydroxyl group.



The mass spectrum of crustecdysone, measured on a Hitachi–Perkin–Elmer RMU-6D spectrometer equipped with a direct evaporative inlet system, has a parent peak at *m/e* 480 and prominent peaks at *m/e* 462, 444, 426, and 408, which are attributed to the loss of one to four molecules of water. The spectrum of ecdysone shows the loss of up to three molecules of water. Major peaks at *m/e* 99 and 81, also present in the spectrum of ecdysone, reveal the same side-chain C-20–C-22 bond fragmentation which occurs in ecdysone (see I). Major peaks in the spectrum of ecdysone at $M-116$ and $M-116-18$ indicate that fission of the side chain takes place by hydrogen-atom transfer, as takes place in the fission of a similar bond in (V).[11] However, major peaks at $M-117$ and $M-117-18$, in the spectrum of crustecdysone indicate cleavage without rearrangement which is expected of a vicinal diol[12,13] and is consistent with the presence in crustecdysone of a 20-hydroxyl group.

Although it is not yet possible to give an unequivocal proof of the structure of crustecdysone, the spectral data clearly indicate the close similarity of the crustecdysone and ecdysone structures and this is further supported by the high biological activity of crustecdysone in the insect test.

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