Structure of Deoxycrustecdysone, a Second Crustacean Moulting Hormone

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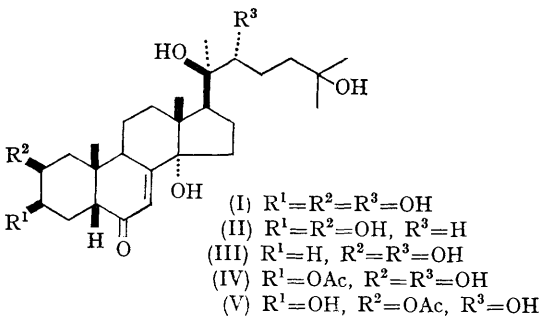
BY fractionating the extract of a larger amount (3 tons) of crayfish waste[1] we have succeeded in isolating, in a substantially pure but noncrystalline form, a small amount (200 μg.) of the moulting hormone less polar than crustecdysone.[2] This new compound has the characteristic u.v. absorption maximum (λ 243 mμ, ϵ *ca.* 7000 in ethanol) of the 7-en-6-one chromophore of the ecdysones and is about as active as crustecdysone in the *Calliphora* test. When first detected,[2] the compound was thought to be ecdysone, but has in fact a higher R_F value (0·30) than ecdysone† (0·23) in thin-layer chromatography.‡ From its mass spectrum, the new compound, named deoxycrustecdysone, has one hydroxy-group less than crustecdysone and the structure (II) (22-deoxycrustecdysone)

† We are indebted to Drs. J. Siddall and P. Hocks for reference samples of synthetic ecdysone.
‡ Unactivated silica gel (Merck type H254) was used with chloroform–96% ethanol (80–20) as solvent.

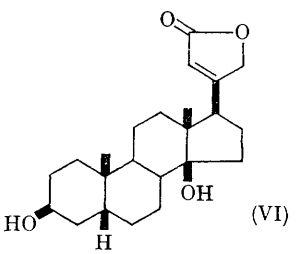
TABLE 1

Chemical shifts of methyl resonances (δ)

	Solvent	C-18	C-19	C-21	C-26–27
Deoxycrustecdysone	..[$^2H_5$]Pyridine	1·21	1·04	1·57	1·35
Crustecdysone ..		1·20	1·07	1·58	1·38
Deoxycrustecdysone	..[$^2H_4$]Methanol	0·93	0·87	1·17	1·17
Crustecdysone ..		0·95	0·88	1·19	1·19



(I) R^1=R^2=R^3=OH
(II) R^1=R^2=OH, R^3=H
(III) R^1=H, R^2=R^3=OH
(IV) R^1=OAc, R^2=R^3=OH
(V) R^1=OH, R^2=OAc, R^3=OH



(VI)

was suggested.[3] We now present evidence for a 2-deoxycrustecdysone structure (III).

In its ^1H n.m.r. spectrum§ deoxycrustecdysone has sharp peaks, attributed to methyl resonances, which are closely similar in chemical shift to those of crustecdysone (Table 1). From these values, deoxycrustecdysone has a structure very similar to that of crustecdysone and hydroxyls are present at C-14, C-20, and C-25. If a hydroxy-group were absent from C-14, the chemical shift of the C-18 methyl resonance would be at higher field,[4] and the biological activity would be expected to be much lower than that of crustecdysone.[5]

The pattern of ions in the high-mass region of the deoxycrustecdysone spectrum (parent ion at *m/e* 464) closely resembles that of crustecdysone with the prominent ions [*m/e* 464 (1), 446 (1), 428 (4), 410 (7), 392 (4), 347 (65), 329 (100), 311 (23), 295 (18), 285 (42)], sixteen mass units less than the corresponding ions in the spectrum of crustecdysone [480 (4), 462 (2), 444 (6), 426 (19), 408 (9), 363 (48), 345 (100), 327 (47), 311 (19), 301 (26)]. The intense peak in the spectrum of deoxycrustecdysone at *m/e* 347 (*M*−117), indicative of ready side-chain cleavage, is attributed, like that in the spectrum of crustecdysone [*m/e* 363 (*M*−117)] to scisson of a C-20–C-22 vicinal diol. This interpretation is supported by the presence of the prominent peaks at *m/e* 99 and 81, assigned as in the case of crustecdysone, to ions produced from the side-chain fragment. It follows that the side chains of deoxycrustecdysone and crustecdysone are the same, and that the deoxycrustecdysone tetracycle has one hydroxy-group less than crustecdysone. The tertiary 14α-hydroxy-group of the ecdysones is chemically labile and the intense peak at *m/e* 414 (*M*—18) in the spectrum of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one[7] is undoubtedly due to the elimination of the 14α-hydroxy-group. As the base peak of the deoxycrustecydsone spectrum occurs at *m/e* 329 (*M*−117−18), that is, 18 mass units less than expected for simple side-chain cleavage, the mass-spectral data provide further evidence for the presence of a 14α-hydroxy-group in deoxy-crustecdysone.

Confirmation of the presence of a single vicinal diol in deoxycrustecdysone at C-20–C-22 was obtained by oxidizing the hormone (10 μg.) with periodic acid (20 μl. of 1%) in ethanol. The reaction product gave a major spot in thin-layer chromatography,‡ with an R_F-value (0·42) similar to that of 2β-acetoxy-3β,14α-dihydroxy-5β-pregn-7-en-6,20-dione (0·51) prepared for comparison.

From acetylation studies, deoxycrustecdysone has two readily acetylated hydroxy-groups, one of which can be assigned to the 22-position. The remaining unassigned hydroxy-group can be accommodated at a number of positions in the tetracycle, but is most probably present as a 2β- or 3β-substituent. As such hydroxy-substituents would differ in orientation (axial and equatorial, respectively) and therefore in reactivity, a study was made of the rates of acetylation at 20° of the hydroxy-groups of deoxy-crustecdysone (30 μg.) and of several model

§ Obtained with a Varian HA-100 spectrometer, equipped with a C-1024 time-averaging computer.

TABLE 2

Rates of acetylation of hydroxy-groups

Compound	Hydroxyl	Rate constant $k \times 10^{-2}$min.$^{-1}$
Deoxycrustecdysone	Ring	0·8
Digitoxigenin (VI)	3β-Axial	0·5
Crustecdysone 2-acetate (IV)	3β-Axial	0·3
Crustecdysone 3-acetate (V)	2β-Equatorial	4·0
3β-Hydroxy-5α-cholestan-6-one ..	3β-Equatorial	2·5

compounds using pyridine–acetic anhydride (2 : 1, 50 μl.). The approximate pseudo-first-order rate constants, calculated from intensities of spots on thin-layer chromatograms run at intervals of time, are reported in Table 2. There it is seen that the rate of acetylation of the ring hydroxyl of deoxycrustecdysone is closer to that expected for an axial hydroxy-group. It is thus likely that the new hormone is 2-deoxycrustecdysone (III), and this assignment is the most probable on biogenetic grounds.

We thank Dr. R. E. Lack for a sample of digitoxigenin.

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