The Catabolism of Crustecdysone in the Blowfly *Calliphora stygia*

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Summary Crustecdysone (I) is catabolised in *Calliphora stygia* partly by C-20–C-22 side-chain scission to 4-hydroxy-4-methyl-pentanoic acid (III).

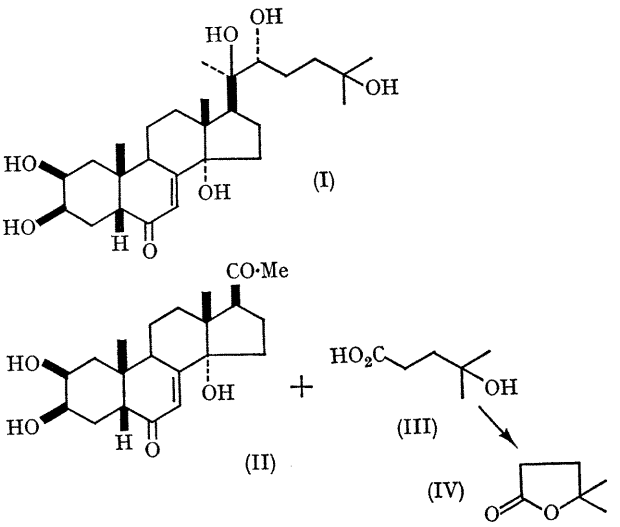
SHAAYA and KARLSON found[1] that in the flowfly *Calliphora erythrocephala* Meig. (*C. vicina* R.-D.) there is a rapid increase in the titre of moulting hormone at the beginning of puparium formation. Recently we showed[2] that the moulting hormone present at this stage is crustecdysone and not ecdysone as believed previously.[3] After puparium formation there is a rapid decrease in moulting hormone titre which is attributed[4] to the appearance of enzymes which catabolise the hormone. As crustecdysone is a 20,22-diol, like postulated intermediates[5] in the biological oxidation of cholesterol, it was suggested[6] that crustecdysone might be catabolised in an analogous way to the 20-ketone (II). Further support for this supposition has now been obtained by a study of the catabolism of [23, 23, 24, 24-$^3H_4$]crustecdysone in prepupae of *Calliphora stygia*.

[23 23, 24, 24-$^3H_4$]Crustecdysone was prepared[7] by injecting similarly labelled α-ecdysone[8] (*ca.* 50c/mmole) into third instar larvae at puparium formation, extracting the prepupae 6 hr. later, and isolating[2] the crustecdysone

without the addition of unlabelled crustecdysone. The activity of the crustecdysone obtained was expected to be somewhat lower (*ca.* 35c/mmole) than that of the injected ecdysone because of dilution with endogenous hormone.

The labelled crustecdysone ($6{\cdot}0 \times 10^7$ d.p.m.) was injected into prepupae 6 hr. after puparium formation and the animals were extracted 6 hr. later. Most of the labelled crustecdysone (>70%) was recovered unchanged. When the labelled crustecdysone was injected into prepupae 20 hr. after puparium formation and the material extracted 6 hr. later, less than 10% of the radioactivity of the extract could be isolated as unchanged crustecdysone, most of the radioactivity being present in the form of water-soluble metabolites which were not readily extracted by butan-1-ol. The sodium salt of 4-hydroxy-4-methylpentanoic acid (III), prepared from 4,4-dimethylbutyrolactone (IV)[9], was added to the aqueous solution of the water-soluble metabolites and the mixture was acidified. Continuous extraction of the mixture with ether recovered the pentanoic acid (III) in the form of its lactone (IV). After fractional distillation of the ether extract with benzene and removal of acids with sodium hydrogen carbonate, the residue was subjected to gas chromatography. The radioactivity appeared as a single peak which coincided with the peak due to 4,4-dimethylbutyrolactone for two different stationary phases (SE 30 and Carbowax 20M). The radioactivity of the lactone fraction accounted for 90% of the volatile constituents of the benzene solution. The identity of the metabolite with 4-hydroxy-4-methylpentanoic acid was further confirmed by conversion into the *p*-bromophenacyl ester. After chromatography, the radioactive fractions were combined and co-crystallized several times with unlabelled *p*-bromophenacyl 4-hydroxy-4-methylpentanoate (m.p. 58—60° from aqueous ethanol, or 73—74° from light petroleum) to constant activity ($1{\cdot}1 \times 10^6$ d.p.m./mmole).

The radioactivity isolated in the form of dimethylbutyrolactone represented only 0·3% of the total activity of the catabolic products and the amount of incorporation of activity was not increased by injecting unlabelled sodium 4-hydroxy-4-methylpentanoate together with the labelled crustecdysone. Thus it appears that catabolism of crustecdysone in *Calliphora* by direct C-20–C-22 bond scission may not be a major metabolic pathway. The 20-ketone (II) which is expected to be produced in this reaction has not so far been isolated[10] from insects and may undergo further rapid oxidation to compounds such as rubrosterone.[11]



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