The Biosynthesis of Ecdysones in the Blowfly *Calliphora stygia*

By J. A. THOMSON
(*Department of Genetics, University of Melbourne, Australia*)
J. B. SIDDALL
(*Zoëcon Corporation, 975 California Avenue, Palo Alto, California, U.S.A.*)
and M. N. GALBRAITH, D. H. S. HORN,* and E. J. MIDDLETON
(*Division of Applied Chemistry, C.S.I.R.O., P.O. Box 4331, G.P.O., Melbourne, Australia*)

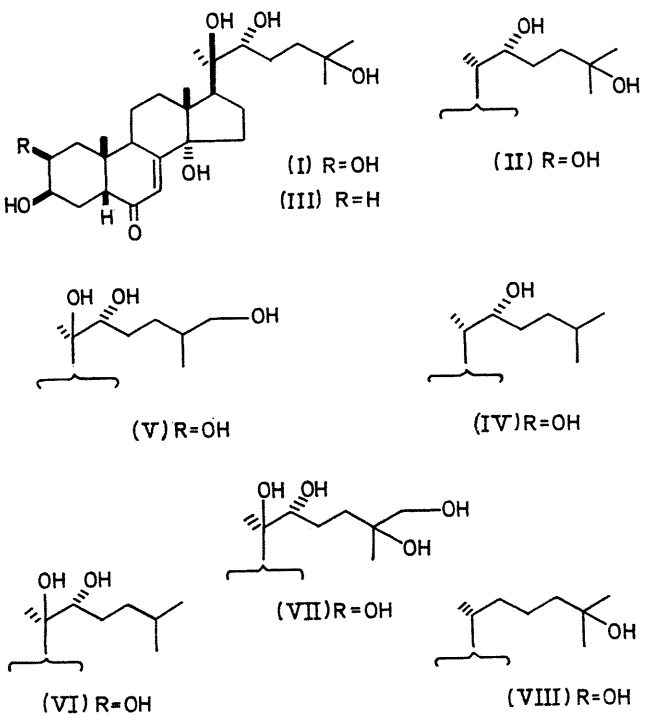
Summary 25-Deoxyecdysone (IV) is metabolised in *Calliphora stygia* to ponasterone A (VI) and inokosterone (V) as well as crustecdysone (I), and is thus unlikely to be a major natural precursor of crustecdysone in this insect.

IN an earlier communication[1] it was established that crustecdysone (I) is the only hormone present in significant amounts in prepupae of *Calliphora stygia* and *C. vicina* and that ecdysone (II), if present, is at a much lower concentration. Recently it was found[2] that tritium-labelled ecdysone injected into third instar larvae of *Calliphora stygia* is rapidly metabolised to a single major product identified as crustecdysone. King and Siddall[3] have also observed the conversion of ecdysone in *Calliphora vicina* and two crustaceans *Crangon* and *Uca*.

The tritium-labelled ecdysone (α-ecdysone) used in these studies was prepared[3,4] by platinum-catalysed tritiation of 2β,3β,14α,22*R*,25-pentahydroxy-5β-cholest-7-en-23-yn-6-one in dry tetrahydrofuran solution using carrier-free tritium gas. Chromatography of the product afforded besides tritiated ecdysone the hydrogenolysis by-product [23,24,24,25,25-$^3H_5$]-25-deoxyecdysone (IV). As 25-deoxyecdysone can be considered as a possible precursor of ecdysones in insects, a study of its metabolism in *Calliphora stygia* has been undertaken.

When tritiated 25-deoxyecdysone (IV) (specific activity 31·5 c/mmole) was injected into third instar larvae of *Calliphora stygia* at the time of puparium formation and the prepupae were extracted 3 hr. later, a complex mixture of metabolites was obtained. The major product, isolated as

described,[1] had the R_F value of crustecdysone on t.l.c. but was separated by column chromatography into fractions with elution volumes corresponding to crustecdysone (I) and inokosterone (V).[5,6] The identity of the compounds was further confirmed as follows: unlabelled crustecdysone was added to the first fraction eluted to give a specific activity of $1{\cdot}3 \times 10^4$ c.p.m./μmole and recrystallized to constant activity ($1{\cdot}3 \times 10^4$ c.p.m./μmole). Partial acetylation[7] and column chromatography of the product afforded unchanged crustecdysone ($1{\cdot}0 \times 10^4$ c.p.m./μmole) and crustecdysone 2-acetate ($1{\cdot}0 \times 10^4$ c.p.m./μmole). Unlabelled inokosterone† was added to the second fraction to give a specific activity of $1{\cdot}3 \times 10^6$ c.p.m./μmole and the mixture partially acetylated. In t.l.c. the radioactivity of the product showed a distribution which corresponded closely with the area and intensity of the u.v.-absorbing spots. The main acetyl derivative present (considered to be the 2,26-diacetate) was isolated by column chromatography and found to have a specific activity of $1{\cdot}3 \times 10^6$ c.p.m./μmole.



The less polar material eluted after crustecdysone and inokosterone in reversed-phase chromatography[1] was separated by column chromatography into unchanged 25-deoxyecdysone (IV) and ponasterone A (VI).[8] Unlabelled ponasterone A (isolated from *Podocarpus neriifolius* leaves[7]) was added to the latter material to give a specific activity of $8{\cdot}8 \times 10^3$ c.p.m./μmole and recrystallised to constant activity ($6{\cdot}6 \times 10^3$ c.p.m./μmole). Partial acetylation and column chromatography afforded ponasterone A 2-acetate ($6{\cdot}4 \times 10^3$ c.p.m./μmole).

Although ponasterone A[6] and inokosterone[8] have been isolated previously from plants this is the first report of their isolation from an insect. Inokosterone has been isolated[5] from the crab *Callinectes sapidus* at premoult stages but was not detected[1] in *Calliphora*. However, it is clear that *Calliphora* possess hydroxylases which can introduce 26- as well as 25-hydroxy-groups in ecdysone analogues. 20,26-Dihydroxyecdysone (VII) has been found[9] to occur in the tobacco hornworm *Manduca sexta* and it can be expected that crustecdysone is further metabolised to this compound in *Calliphora*.

Surprisingly, labelled ecdysone could not be detected among the less polar 25-deoxyecdysone metabolites. However, it is possible that ecdysone is a metabolite and that the rate of hydroxylation of 25-deoxyecdysone is slower than that at C-20; so that ponasterone A is produced faster than ecdysone but is more slowly converted to crustecdysone than ecdysone. Ponasterone A would then be expected to accumulate while ecdysone would be consumed as fast as it is produced. Labelled crustecdysone and inokosterone are present in equal concentrations. The rate of hydroxylation of ponasterone A at C-26 is thus probably the same as that at C-25.

As ponasterone A and inokosterone were not detected[1] in significant amounts in extracts of *Calliphora* it is concluded that 25-deoxyecdysone is not a normal major precursor of crustecdysone in *Calliphora*. Also it is unlikely that 22-deoxyecdysone (VIII) can be a precursor of crustecdysone because it would be expected to afford 22-deoxycrustecdysone,[10] which was not detected[1] in *Calliphora stygia*. In addition, 22-deoxycrustecdysone has been shown[10] to have low biological activity in the *Calliphora* bioassay. Thus it is likely that in the biosynthesis of crustecdysone in *Calliphora*, side-chain hydroxylation of precursor sterols at C-22 and C-25 precedes elaboration of the tetracycle. However, in plants which contain ponasterone A and inokosterone, 25-deoxyecdysone may well be a precursor of these compounds. In the crayfish *Jasus lalandei* deoxycrustecdysone (III)[11] with a fully hydroxylated side chain can be expected to be the precursor of crustecdysone.

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[1] M. N. Galbraith, D. H. S. Horn, J. A. Thomson, G. J. Neufeld, and R. J. Hackney, *J. Insect Physiol.*, 1969, in the press.
[2] D. H. S. Horn, E. J. Middleton, and J. A. Thomson, unpublished work.
[3] D. S. King and J. B. Siddall, *Nature*, 1969, **221**, 955.
[4] G. Hüppi and J. B. Siddall, *Tetrahedron Letters*, 1968, 1113.
[5] A. Faux, D. H. S. Horn, E. J. Middleton, H. M. Fales, and M. E. Lowe, *Chem. Comm.*, 1969, 175.
[6] T. Takemoto, S. Ogawa, and N. Nishimoto, *J. Pharm. Soc. Japan*, 1967, **87**, 325.
[7] M. N. Galbraith and D. H. S. Horn, *Austral. J. Chem.*, 1969, in the press.
[8] K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, and H. Y. Hsu, *Chem. Comm.*, 1966, 915.
[9] M. J. Thompson, J. N. Kaplanis, W. E. Robbins, and R. T. Yamamoto, *Chem. Comm.*, 1967, 650.
[10] M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Chem. Comm.*, 1968, 466.
[11] M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and R. J. Hackney, *Chem. Comm.*, 1968, 83.