Ecdysone Biosynthesis in the Blowfly *Calliphora stygia*

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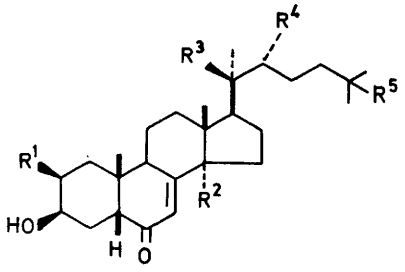
Summary 3β,14α-Dihydroxy-5β-[3α-^{3}H]cholest-7-en-6-one (**1**) is metabolised in *Calliphora stygia* at the time of puparium formation *via* hydroxylated derivatives to α-ecdysone (**2**) and β-ecdysone (**3**) and thus may be a precursor of ecdysones in this insect.

RECENTLY we reported[1] that the simple ecdysone analogue 3β,14α-dihydroxy-5β-cholest-7-en-6-one (**1**), m.p. 191—192°, is highly active in the *Calliphora* test[2] and suggested this ketone as a possible precursor of moulting hormones in *Calliphora*. We now report on its metabolism in *Calliphora stygia*. For this study the ketone (**1**) was labelled with tritium in the 3α-position by the following series of steps: 5α-cholest-7-ene-3β,6α-diol 6-monoacetate prepared by partial acetylation of the corresponding diol[3] was oxidised with Jones' reagent to the corresponding 3-ketone which was reduced with sodium borotritide (250 mCi, 3 Ci/mmol) mainly to the [3α-^{3}H],3β-hydroxy-epimer. Selective oxidation of this diol with manganese dioxide in chloroform afforded 3β-hydroxy-5α-[3α-^{3}H]cholest-7-en-6-one which

was oxidised with selenium dioxide[4] to the corresponding 14α-hydroxy-derivative. This was treated with potassium carbonate in aqueous methanol to yield an equilibrium mixture from which 3β,14α-dihydroxy-5β-[3α-^{3}H]cholest-7-en-6-one (**1**) was isolated by chromatography on alumina.

The labelled ketone (**1**) (29×10^6 c.p.m., 0·2 Ci/mmol) was injected into third instar larvae of *Calliphora stygia* at the time of puparium formation and the prepupae extracted[5] 12 h later. From this extract the following fractions were obtained by column chromatography:[6] unchanged material (**1**) (1×10^6 c.p.m.), monohydroxylated metabolites ($3{\cdot}2 \times 10^5$ c.p.m.), 22-deoxy-α-ecdysone (**4**) ($1{\cdot}6 \times 10^5$ c.p.m.), α-ecdysone (**2**) ($1{\cdot}0 \times 10^5$ c.p.m.), and β-ecdysone (**3**) ($1{\cdot}4 \times 10^5$ c.p.m.). The identity of the β-ecdysone was confirmed by dilution of a portion with unlabelled β-ecdysone to a specific activity of $6{\cdot}67 \times 10^5$ c.p.m/mmol and recrystallization from MeOH–EtOAc. The specific activity of the mixture was unchanged after three crystallizations (5·86, 6·06, and $6{\cdot}27 \times 10^5$ c.p.m./mmol, respectively).

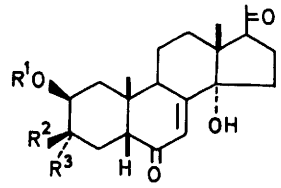
To confirm that the label in the isolated β-ecdysone was confined to the C-3 position, diluted β-ecdysone ($7{\cdot}45 \times 10^5$ c.p.m./mmol) was oxidized with CrO_3–C_5H_5N to the pregnane derivative (**8**) (specific activity $7{\cdot}53 \times 10^5$ c.p.m./



	R^1	R^2	R^3	R^4	R^5
(**1**)	H	OH	H	H	H
(**2**)	OH	OH	H	OH	OH
(**3**)	OH	OH	OH	OH	OH
(**4**)	OH	OH	H	H	OH
(**5**)	H	OH	H	H	OH
(**6**)	H	H	H	H	H
(**7**)	OH	OH	H	H	H

mmol). Brief acetylation[6] gave the corresponding 2-acetate (**9**) (specific activity $7{\cdot}10 \times 10^5$ c.p.m./mmol) which on oxidation with Ac_2O–Me_2SO overnight[7] at 20° afforded the corresponding 3-ketone (**10**), m.p. 206—209°, with specific activity of $0{\cdot}16 \times 10^5$ c.p.m./mmol. Thus 98% of the tritium label in the metabolite is in the 3α-position.

α-Ecdysone (**2**) was shown to be present by chromatography in several systems, and by dilution with unlabelled α-ecdysone to a specific activity of $5{\cdot}00 \times 10^5$ c.p.m./mmol and recrystallization to constant activity (4·75, 4·45, and 4·37 × 10^5 c.p.m./mmol). The labelled 22-deoxy-α-ecdysone was identified by column chromatography with unlabelled material before and after brief acetylation.[6] The peaks of radioactivity of the two main acetates in the mixture correspond in proportion and elution volume,



	R^1	R^2	R^3
(**8**)	H	OH	^{3}H
(**9**)	Ac	OH	^{3}H
(**10**)	Ac	=O	

when allowance is made for the isotope effect,[8] to the u.v. peaks due to 22-deoxy-α-ecdysone 2,3-diacetate and 2-monoacetate. The monohydroxylated metabolite fraction consists of a complex mixture. From acetylation studies the main component is tentatively assigned structure (**5**) with an additional tertiary hydroxy-group. The main pathway in the metabolism of the ketone (**1**) to β-ecdysone may thus be (**1**)→(**5**)→(**4**)→(**2**)→(**3**). This conclusion is further supported by the observations that α-ecdysone (**2**)[9] and 22-deoxy-α-ecdysone (**4**)[10] are metabolised to β-ecdysone (**3**) in *Calliphora* at this stage. Earlier it was shown[10] that the triol (**7**) can serve as a precursor of β-ecdysone in *Calliphora* and it has been suggested as a normal precursor of ecdysones in *Manduca sexta*.[11] However, this compound, if present in the mixture of metabolites of the ketone (**1**), was at a very much lower concentration than the isomeric triol (**5**) and is not a major metabolite. The ketone (**1**) is incorporated in good yield (0·5%) into β-ecdysone and thus may be a precursor of ecdysones in *Calliphora*.

The labelled ketone (**1**) was similarly converted into α- and β-ecdysones in isolated *Calliphora* abdomens demonstrating the competence of these tissues in the absence of ring gland to undertake these metabolic steps. The [3α-^{3}H]-5β-ketone (**6**), prepared by equilibration of the 5α-ketone and column chromatography, was also tested in intact *Calliphora stygia* as a precursor of ecdysones but it was not incorporated into either β-ecdysone or the ketone (**1**), and is thus unlikely to be an intermediate in the biosynthesis of ecdysones from cholesterol.

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