Possible Biosynthetic Precursors of β-Ecdysone in *Calliphora stygia*

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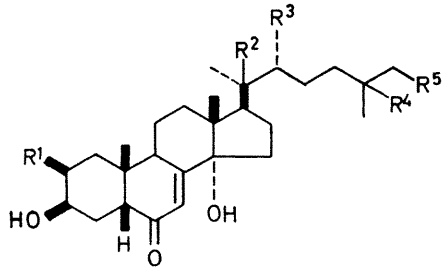
Summary 22-Deoxy-α-ecdysone and 22,25-dideoxy-α-ecdysone are rapidly metabolised in *Calliphora stygia* to β-ecdysone but may not be natural precursors of this insect hormone.

IN an earlier study[1] it was found that 25-deoxy-α-ecdysone (**1**) is metabolised in *Calliphora stygia* to β-ecdysone (**2**) (crustecdysone) and a number of other ecdysone analogues [*viz.* ponasterone A (**3**) and inokosterone (**4**)] which are not normally present in *C. stygia*.[2] It was thus concluded that 25-deoxy-α-ecdysone (**1**) cannot be a normal precursor of β-ecdysone (**2**) in this insect. To obtain further information about the biosynthesis of β-ecdysone (**2**) in *C. stygia* a study has now been made of the metabolism of tritium-labelled 22-deoxy-α-ecdysone (**5**) and 22,25-dideoxy-α-ecdysone (**6**).

The steroids[3] were labelled by 10% palladium–charcoal-catalysed hydrogenation of the allene 2β,3β,14α,25-tetrahydroxy-5β-cholesta-7,22,23-trien-6-one in ethanol using diluted tritium gas. Chromatography of the product afforded [22,23,24-^{3}H]-2β,3β,14α,25-tetrahydroxy-5β-cholest-7-en-6-one [22-deoxy-α-ecdysone (**5**), specific activity 42 Ci/mmole] and [22,23,24,25-^{3}H]-2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one [22,25-dideoxy-α-ecdysone (**6**), specific activity 106 Ci/mmole]. When the diluted tritium-labelled 22-deoxy-α-ecdysone (**5**) (50 μCi, specific activity 7 Ci/mmole) was injected into 3rd instar larvae of *C. stygia* at the time of puparium formation and the prepupae extracted 3 h later, about 60% of the injected steroid was metabolised to more polar products, 25% of which was identified as β-ecdysone (**2**). A portion of the purified β-ecdysone isolated was mixed with unlabelled β-ecdysone to give a specific activity of $10{\cdot}3 \times 10^6$ d.p.m./mmole and recrystallised from methanol–ethyl acetate. The specific activity of the mixture was constant after three crystallisations (11·1, 11·3, and $11{\cdot}1 \times 10^6$ d.p.m./mmole). A minor metabolite (5% of the total metabolites) was identified as α-ecdysone (**7**). Cocrystallization of the material with unlabelled α-ecdysone afforded after three crystallisations a product of constant specific activity ($4{\cdot}2 \times 10^6$ d.p.m./mmole), which on acetylation and recrystallisation afforded α-ecdysone triacetate with a specific activity of $4{\cdot}2 \times 10^6$ d.p.m./mmole. Most of the remaining unidentified metabolites were more polar than β-ecdysone (**2**) and are probably formed by catabolic processes.[4]



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

When 22,25-dideoxy-α-ecdysone (**6**) (18 μCi, 10·6 Ci/mmole) was incubated in *C. stygia* for 3 h, about 60% was

metabolised to more polar compounds which were identified by chromatography as 22-deoxy-α-ecdysone (**5**) (25%), α-ecdysone (**7**) (2%), and β-ecdysone (**2**) (4%). The bulk of the remaining metabolites again consisted of substances more polar than β-ecdysone. Neither ponasterone A (**3**) nor inokosterone (**4**) was present in the mixture of metabolites.

α-Ecdysone (**7**) was formed in significant amounts from both 22-deoxy-α-ecdysone (**5**) and 22,25-dideoxy-α-ecdysone (**6**), whereas α-ecdysone (**7**) could not be isolated from a large scale extract of *C. stygia* harvested at the time of puparium formation.[2] Thus it appears that these compounds may not be normal precursors of β-ecdysone in *C. stygia*. Instead it is possible that the biosynthesis proceeds through 2-deoxy-α-ecdysone precursors since both deoxycrustecdysone (**8**)[5] and deoxyecdysone (**9**)[6] are highly active in the *Calliphora* test and are thus presumably efficiently metabolised to β-ecdysone. King and Siddall[7] have found that 22,25-dideoxy-α-ecdysone is converted efficiently into 22-deoxy-α-ecdysone, α-ecdysone, β-ecdysone, and 26-hydroxy-β-ecdysone by *Manduca sexta* prepupae *in vivo* and by isolated fat body and malpighian tubules *in vitro*, but that metabolism of compound (**6**) in *Sarcophaga*, *Gastrimargus*, and *Dermestes* leads to very complex mixtures of polar steroids containing little (<5%) or no α- or β-ecdysone. Kaplanis *et al.*[8] found that 22,25-dideoxy-α-ecdysone is converted by *Manduca sexta* into α- and β-ecdysone and concluded that this triol is probably an intermediate in the biosynthesis of ecdysones in *M. sexta*.

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