Fate of the C-4 Hydrogen Atoms of Cholesterol during its Transformation into Ecdysones in Insects and Plants

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Summary Transformation of synthetic [4α-^{3}H]- and [4β-^{3}H]-cholesterols into ecdysones in the insect, *Calliphora erythrocephala* involves elimination of the 4β hydrogen, whereas in the plant, *Polypodium vulgare* both hydrogen atoms are retained.

The saturation of the 5,6-double bond of cholesterol to give a 5β stereochemistry during its metabolic transformation into insect moulting hormones (ecdysones) could occur *via* numerous pathways.[1] An analogous change during steroid hormone and bile acid[2] formation in animals involves the intermediacy of a 3-oxo-Δ^4-steroid. Similarly the introduction of 5β stereochemistry of cardenolides in *Digitalis lanata* requires oxidation at C-3 to be an obligatory step.[3]

In order to evaluate the possibility of a similar transformation during metabolic conversion of cholesterol into ecdysones, [4α-^{3}H]- and [4β-^{3}H]-cholesterol were synthesised and separately administered, together with [4-^{14}C]-cholesterol to the plant, *Polypodium vulgare* and to the insect, *Calliphora erythrocephala.*

[4β-^{3}H]Cholesterol was prepared by the method of Ireland, Wrigley, and Young.[4] [4α-^{3}H]cholesterol was prepared in five steps from cholest-5-en-3-one (**8**). Acetoxylation with $Pb(OAc)_4$ yielded the 4-acetoxy-derivative (**9**) which upon isomerisation over alumina[5] gave 3β-acetoxycholest-5-en-4-one (**10**). Reduction of the 4-oxo-group with $NaBT_4$ yielded the 4α (**11b**) and 4β (**11a**) epimeric alcohols. The 4β-epimer was separated by t.l.c. and converted into the 6-chloro-4-ene derivative (**12**) by brief reaction with thionyl chloride.[4] Reduction of (**12**) with $LiAlH_4$ yielded [4α-^{3}H]cholesterol.

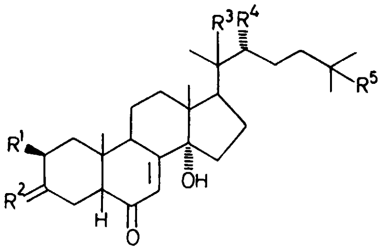
The radiochemical purity of the synthetic cholesterols was ascertained by allylic 4β-hydroxylation of the derived benzoates,[6] followed by chromic acid oxidation.[7] By this method 91% of the tritium in both [4α-^{3}H]- and [4β-^{3}H]-cholesterol was shown to be at the expected position.

In the case of *P. vulgare*, administration of radioactive cholesterol, extraction, and purification of ecdysone (**1**) and ecdysterone (**2**) were carried out essentially as previously described.[8] Radioactive cholesterol was administered to *Calliphora* larvae by injection, as a suspension in insect Ringer's solution containing Tween 20. After extraction with methanol and chloroform, the combined extracts were evaporated and partitioned between n-butanol and water. The butanol extract was evaporated and re-partitioned between hexane and 70% aqueous methanol. Chromatography of the hexane fraction yielded recovered cholesterol, and t.l.c. of the methanolic fraction gave ecdysone and ecdysterone.

TABLE

^{3}H : ^{14}C Atomic ratios (based on administered cholesterol) of the steroids (and their derivatives) isolated from *P.vulgare* and *C. erythrocephala* after administration of [4β-^{3}H,4-^{14}C]- and [4α-^{3}H,4-^{14}C]-cholesterol.

Administered substrate	[4β-^{3}H,4-^{14}C]-Cholesterol		[4α-^{3}H,4-^{14}C]-Cholesterol	
Approx. amount	40 μCi ^{3}H, 20 μCi^{14}C	80 μCi^{3}H, 40 μCi^{14}C	50 μCi^{3}H, 20 μCi^{14}C	130 μCi^{3}H, 40 μCi^{14}C
Organism	*P. vulgare*	*C. erythrocephala*	*P. vulgare*	*C. erythrocephala*
Experiment No.	1	2	3	4
Compound				
Administered cholesterol	1·00 : 1	1·00 : 1	1·00 : 1	1·00 : 1
Recovered cholesterol	1·09 : 1	0·96 : 1	1·08 : 1	1·12 : 1
2-Acetoxyecdysone (**3**)	0·99 : 1	0·04 : 1	1·05 : 1	
Tetra-acetoxyecdysone (**6**)				0·95 : 1
2-Acetoxyecdysterone (**4**)			0·92 : 1	
2-Acetoxy-20,22-acetonidecdysterone (**5**)	0·97 : 1	0·06 : 1		1·04 : 1
2-Acetoxy-3-oxo-20,22-acetonidecdysterone (**7**)	0·92 : 1			0·90 : 1

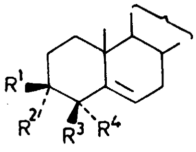


(**1**) R^{1}=R^{4}=R^{5}=OH, R^{2}= β-OH,α-H, R^{3}=H
(**2**) R^{1}=R^{3}=R^{4}=R^{5}=OH, R^{2}= β-OH, α-H
(**3**) R^{1}=OAc, R^{2}= β-OH, α-H, R^{3}=H, R^{4}=R^{5}=OH
(**4**) R^{1}=OAc, R^{2}= β-OH, α-H, R^{3}=R^{4}=R^{5}=OH
(**5**) R^{1}=OAc, R^{2}= β-OH, α-H, R^{3}, R^{4}= –OCMe$_2$O–, R^{5}=OH
(**6**) R^{1}=R^{4}=R^{5}=OAc, R^{2}= β-OAc, α-H, R^{3} = H
(**7**) R^{1}=OAc, R^{2}=O, R^{3}, R^{4}=–OCMe$_2$O–, R^{5}=OH

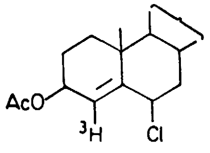
Ecdysones from both sources were purified by t.l.c.; the incorporations from [^{14}C]cholesterol at this stage in *C. erythrocephala* and *P. vulgare* were *ca.* 0·038 and 0·037%, respectively. Further purification was achieved by formation of derivatives,[8,9] which were recrystallised to constant specific radioactivity. The results obtained are in the Table.

Whilst [4α-^{3}H]cholesterol yields ecdysterone and ecdysone with retention of the label in both *P. vulgare* and *C. erythrocephala*, [4β-^{3}H]cholesterol yields ecdysones with retention of label in *P. vulgare* but not in *C. erythrocephala*.

The results with *Calliphora* could be rationalised if a Δ^{4} steroid intermediate were involved, or if the biosynthesised ecdysones were oxidised to the corresponding 3-oxo-derivatives,[10] equilibration of which could result in stereospecific loss of the 4β tritium atom. Retention of the 4α-tritium and the 4β-tritium in the ecdysones produced by *P. vulgare* suggests that the C-4 position is not involved in the biosynthetic transformation in this case. However, it is possible that the 4β-^{3}H is eliminated as in *Calliphora*, and subsequently re-incorporated at C-4 or another position. Previous work has demonstrated that tritium removed from the steroid skeleton during metabolic transformations can be re-introduced at the C-3[9] and C-7[11] position, probably *via* a compartmentalised pool of NADPH. Accordingly, the tritiated 2-acetoxy-20,22-acetonide ecdysterone (**5**) samples from *P.vulgare* and *C. erythrocephala* were oxidised with active MnO$_2$[12] in MeCN to give the 3-oxo-derivative (**7**) in good yield and without acyl migration.[9] Oxidation of (**5**), from experiments 1 and 4, by this method for up to 2 h led to retention of the tritium label in the 3-oxo derivative. However, if the reactions were continued for *ca.* 1 week extensive loss of tritium occurred. Moreover, when the 3-oxo derivative (**7**) was isolated after 2 h and re-submitted to the reaction conditions, further loss of tritium occurred. This behaviour suggests a slow equilibration of (**7**) leading to loss of label from position 4. The results therefore indicate the absence of tritium from C-3



(**8**) R^{1}, R^{2}=O, R^{3}=R^{4}=H
(**9**) R^{1}, R^{2}=O, R^{3}=H, R^{4}=OAc
(**10**) R^{1}=OAc, R^{2}=H, R^{3}, R^{4}=O
(**11a**) R^{1}=OAc, R^{2}=H, R^{3}=OH, R^{4}=^{3}H
(**11b**) R^{1}=OAc, R^{2}=H, R^{3}=^{3}H, R^{4}=OH



(**12**)

and its probable location at C-4. However, in the presence of a 6-oxo-group, re-introduction of the label at C-5 cannot be excluded. On this evidence it is tempting to suggest that the mechanism involved in converting cholesterol into ecdysones is different in *P. vulgare* and *C. erythrocephala*, but this could only be proved in enzyme systems in which reintroduction of tritium *via* NADPH is eliminated.

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