Biosynthesis of Phytoecdysone: Incorporation of 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one into β-Ecdysone and Inokosterone in *Achyranthes fauriei*

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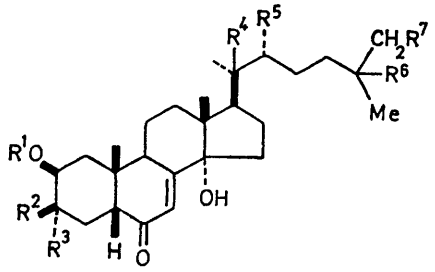
Summary 2β,3β,14α-Trihydroxy-5β-[3α-$^3H_1$]cholest-7-en-6-one was incorporated into both β-ecdysone and inokosterone, equally; thus hydroxylation of the side chain of phytoecdysone occurs after formation of the A–B ring system.

A NUMBER of closely related compounds possessing insect moulting hormone activity are widely distributed in the plant kingdom.[1] Though studies on the biosynthesis of phytoecdysone have been carried out[2] and some precursors for ecdysone in insects have been reported,[3] the detailed biosynthetic pathway has not yet been elucidated.

We report here the incorporation of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (**1**) into β-ecdysone (**2**) and inokosterone (**3**). For this study compound (**1**) was labelled with tritium in the 3α-position in the following way,

since retention of the tritium in biosynthesis can be expected after formation of the A–B *cis* ring system.



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
(2)	H	HO	H	OH	OH	OH	H
(3)	H	HO	H	OH	OH	H	OH
(8)	H	HO	^{3}H	H	H	H	H
(9)	Ac	HO	^{3}H	$(Me)_2C<^{O-}_{O-}$		OH	H
(10)	Ac	HO	H	$(Me)_2C<^{O-}_{O-}$		OH	H
(11)	Ac	H	OH	$(Me)_2C<^{O-}_{O-}$		OH	H

2β-Acetoxy-3β-hydroxy-5α-cholest-7-en-6-one[4,5] (**4**) was oxidized with Jones' reagent to give the corresponding ketone (**5**) which was reduced with tritiated sodium borohydride (1 equiv.) in ethanol to the corresponding alcohol. 2β-Acetoxy-3β-hydroxy-5α-[3α-$^3H_1$]cholest-7-en-6-one (**6**) was isolated in pure form by repeated preparative t.l.c. on silica gel plates (developed with benzene–acetone, 4:1). Oxidation of the acetate of (**6**) with selenium dioxide[4] at 80° in dioxan gave 2β,3β-diacetoxy-14α-hydroxy-5α-[3α-$^3H_1$]-cholest-7-en-6-one (**7**). Treatment of (**7**) with K_2CO_3 in aqueous MeOH yielded a mixture of the 5β-compound (**8**) and its 5α-isomer; compound (**8**) was isolated in pure form by preparative t.l.c. on silica gel plates[4,5] ($CHCl_3$–EtOH, 10:1). A solution of (**8**) ($28{\cdot}9 \times 10^6$ d.p.m.) in acetone was applied to young leaves of *Achyranthes fauriei*. After one week the leaves were harvested, washed with acetone to recover unchanged material ($17{\cdot}16 \times 10^6$ d.p.m.), and then extracted with boiling water. The aqueous solution was extracted with n-butanol and β-ecdysone and inokosterone were isolated as their acetates.[6] The acetates were recrystallized from n-hexane–EtOAc to constant specific radioactivity after addition of carrier compounds: β-ecdysone triacetate $1{\cdot}78 \times 10^6$ d.p.m./mmol (incorporation 0·055%), inokosterone tetra-acetate $1{\cdot}69 \times 10^6$ d.p.m./mmol (incorporation 0·024%). For determination of the location of the tritium label, the β-ecdysone obtained was converted into (**9**),[7,8] which was oxidized with Jones' reagent to yield a mixture of ketones.[8] These were reduced with sodium borohydride to the corresponding alcohols, and (**10**) and its 3-epimer (**11**) were isolated in pure form by repeated preparative t.l.c. (benzene–EtOAc, 6:4): (**10**) $0{\cdot}29 \times 10^5$ d.p.m./mmol, (**11**) $0{\cdot}37 \times 10^5$ d.p.m./mmol. Thus *ca.* 80–84% of the tritium was in the 3α- position of β-ecdysone. These results indicate that 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one is a precursor for β-ecdysone and inokosterone, and that hydroxylation of the side chain occurs after formation of the A–B ring system in phytoecdysone biosynthesis.

(*Received*, 13*th March* 1974; *Com.* 289.)

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