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Synthesis of a Tritiated 3-Dehydroecdysteroid Putative Precursor of Ecdysteroid Biosynthesis in *Locusta migratoria*

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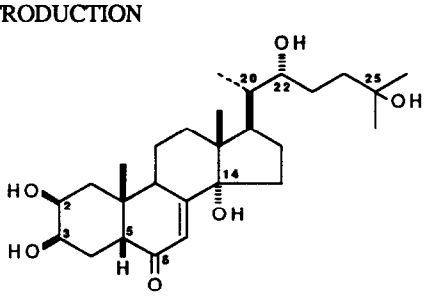
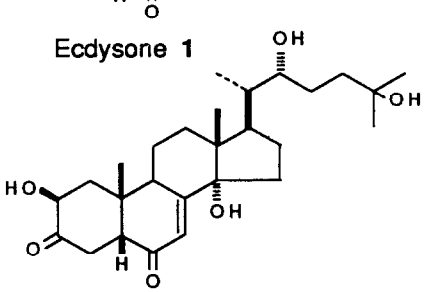
Abstract - We have synthesized a tritiated form of 14α-hydroxy-5β-cholest-7-ene-3,6-dione (5β-diketol) of high specific activity (1 74 TBq/mmol) from 7-dehydrocholesterol in seven steps with stereoselective introduction of the A/B cis ring junction as the key reaction We have examined the ability of endocrine glands (prothoracic glands) of *Locusta migratoria* to use this molecule as a precursor of 3-dehydroecdysone and of ecdysone biosynthesis A very efficient conversion of 5β-diketol to ecdysone and to 3-dehydroecdysone was monitored, which opens up new possibilities for the understanding of the role of 3-dehydro-compounds in ecdysteroid biosynthesis

INTRODUCTION

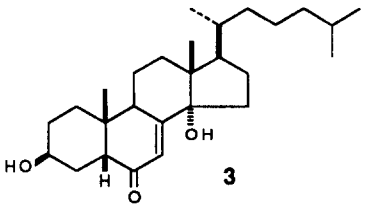


Ecdysone 1



3-Dehydroecdysone 2

Ecdysteroids represent a family of polyhydroxylated steroids which serve as hormonal messengers in insects The parent molecule, ecdysone (**1**), which is mostly referred to as the molting and metamorphosis hormone, was considered as the hormone synthesized and secreted during postembryonic development by endocrine glands (known as prothoracic glands or homologous structures) Recently it has been reported that, in some insect species, prothoracic glands secrete 3-dehydroecdysone (**2**) *in vitro* in addition to ecdysone (**1**)[1,2] This molecule is rapidly reduced to ecdysone by a hemolymph reductase which exclusively reduces the keto group to 3β-hydroxyl[3] In ecdysone biosynthesis investigations, 3-oxo compounds have never been identified, but the precursors used for these studies contained a C-3β hydroxy group[4]



3

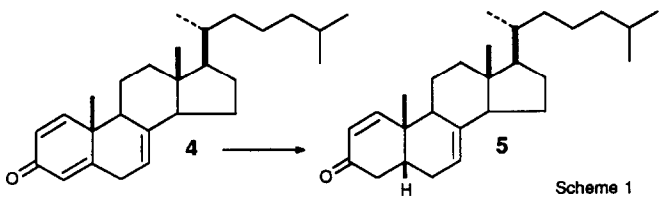
These data prompted us to undertake the synthesis of 14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17**, 5β-diketol) in labelled form This molecule is related to 5β-ketodiol **3** (3β,14α-dihydroxy-5β-cholest-7-en-6-one), which is a well established precursor in ecdysone biosynthesis[5]

The instability of the A/B cis ring junction (5β-H configuration) due to the absence of a 2β-hydroxy group in the target molecule and the opportunity for the stereoselective hydrogenation of the Δ^4 double bond which will give the desired 5β configuration, led us to develop a novel route for the synthesis of 2-deoxyecdysteroids

The strategy which we propose in the present paper for the synthesis of labelled 5β-diketol **17** is firstly the hydrogenation of a Δ^4 double bond to give the A/B cis ring junction and secondly the labelling of the molecule at the last step by tritiation of a Δ^1 double bond.

SYNTHESIS OF 14α-HYDROXY-5β-CHOLEST-7-ENE-3,6-DIONE

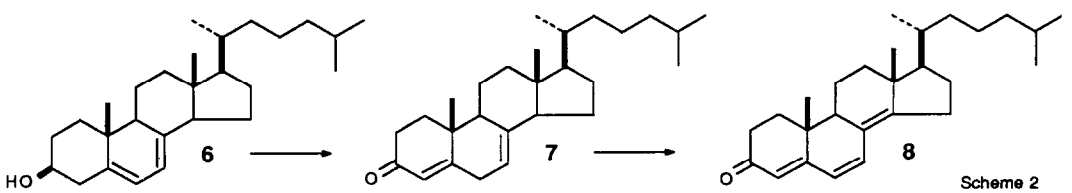
The key compound of our synthesis is **5**, which has all the structural features required to lead to the target molecule **17** . i) the A/B cis ring junction is indispensable for the biological activity, ii) the Δ^7 double bond has two allylic sites C-6 and C-14 which can be efficiently oxidized to the conjugated hydroxy enone system; iii) the Δ^1 double bond can be specifically reduced with tritium at the last step



Scheme 1

It was likely that compound **5** could be obtained readily from **4** by selective reduction of Δ^4 double bond without any reaction on Δ^1 or Δ^7 double bonds, as described by Rubin and Armbrecht[6] (Scheme 1)

Thus we planned to synthesize compound **4** directly from 7-dehydrocholesterol (**6**) in a few steps (Scheme 2) The dienone **7** is obtained by Oppenauer oxidation[7] in good yield (85%)

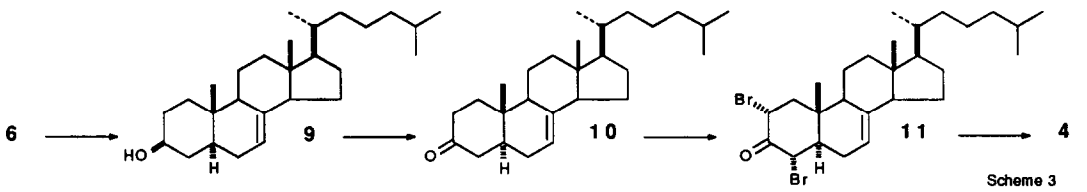


Scheme 2

Treatment of **7** with DDQ (2,3-dichloro-5,6-dicyano-benzoquinone)[8,9] in refluxing dioxan, unfortunately produced the polyenone **8** in 55% yield instead of the expected product **4**

This compound **8** was also obtained during the attempted bromination of **7** with tri-N-methylanilinium perbromide[10] due to spontaneous dehydrobromination

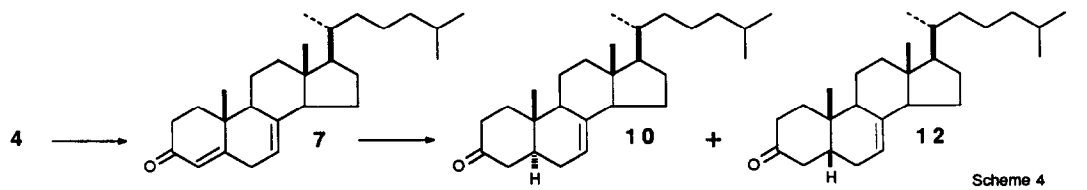
We overcame this difficulty by using a longer synthetic pathway (Scheme 3) 7-Dehydrocholesterol (**6**) was easily reduced by catalytic hydrogenation with palladium on charcoal. Lathosterol (**9**), which was obtained with a very good yield (overall 98%), was oxidized with PCC (pyridinium-chlorochromate) adsorbed on alumina[11] This technique is very convenient because PCC can be easily eliminated by filtration and the yield was particularly high (95% for compound **10**) The ketone **10** was brominated with two equivalents of tri-N-methylanilinium perbromide in THF at 0°C Recrystallization gave the pure dibromide in 64% yield The dibromide **11** was fully characterized and the ^{1}H NMR spectrum indicated that the bromine atoms occupied the α positions (^{1}H NMR δ = 4 67 ppm, d, J = 13 2 Hz, 1H, H-4$_{ax}$ and δ = 4 87 ppm, dd, J = 5 8 and 14 2 Hz, 1H, H-2$_{ax}$) Dehydrobromination[12] in refluxing DMF with LiBr and $LiHCO_3$ afforded the trienone **4** (90%)



Scheme 3

Compound **4** was submitted to the well described reduction[6] with palladium on calcium carbonate in the presence of quinoline and ethanol and afforded a mixture of **7**, **10** and **12**

It is clear that the Δ^1 double bond was first reduced, followed by reduction of the Δ^4 double bond which gave the ketones **10** and **12** (Scheme 4). The Δ^7 double bond never reacts under these conditions Similarly, reduction with palladium on charcoal (with or without quinoline), with other catalysts and in various solvents, led first to the hydrogenation of the Δ^1 double bond. A recent publication[13] confirmed this disappointing result.



Scheme 4

In consequence we have devised an original regio- and stereoselective synthetic pathway to obtain compound **5** (Scheme 5)

Compound **7** was directly reduced to give the A/B cis ring junction with palladium on charcoal in a mixture of aqueous NaOH 0 1N and ethanol (85%)[14-16] The reduction was regioselective, only the Δ^4 double bond being hydrogenated, and stereoselective, the junction of the A/B ring being in cis configuration (H in 5β) with 82% diastereoisomeric excess The A/B cis ring junction was confirmed by ^{1}H and ^{13}C NMR (^{1}H NMR δ = 0 99 ppm (CH_3-19, cis), δ = 1 01 ppm (CH_3-19, trans), ^{13}C NMR δ = 23 5 ppm, 33 9 ppm and 42 0 ppm (C-19, C-9 and C-5 cis), δ = 12 5 ppm, 49 0 ppm and 43 0 ppm (C-19, C-9 and C-5 trans), For references, see Table 1)

The bromination with tri-N-methylanilinium perbromide yielded the 2β bromoderivative **13** (80%) Attempted recrystallization of an aliquot from methanol/ether gave the bromo-ketal **14** The ^{1}H NMR spectrum indicated that the bromine atom occupied the 2β position (^{1}H NMR . δ = 4 34 ppm, dd, J = 4 5 Hz and 13 0

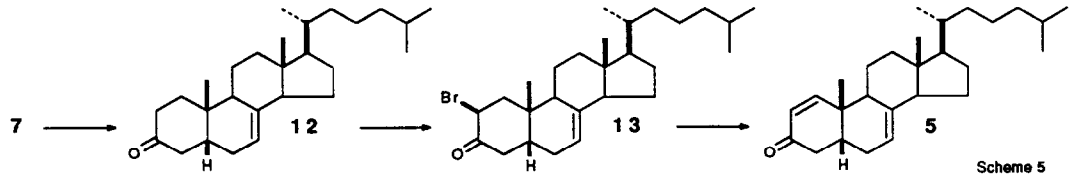
	4	5	7	8	9	10	11	12	14	15	16	17	18
1	155 0*	162 2*	33 4*	39 4*	31 6*	38 2*	50 9	35 6*	36 9*	157 7*	159 9*	31 2*	152 6*
2	127 9*	127 8*	34 2*	34 1*	37 3*	39 6*	52 2*	37 8*	39 5*	128 8*	128 8*	34 9*	129 2*
3	186 1	200 5	199 0	199 5	71 3	211 8	193 0	212 3	99 2	198 0+	198 7+	208 3	188 1+
4	123 5*	40 5	122 8	122 9	39 6*	44 3*	51 5*	43 6*	45 5*	35 7	36 3	37 0*	126 6*
5	165 9	40 2	168 6	164 5+	40 4	43 0	59 5*	42 0	55 0	52 0	52 0	55 3	157 8'
6	32 7	27 8	32 9*	124 4'	29 8	30 2	29 6	28 5	33 8	196 4+	196 3+	199 4	185 6+
7	115 8	115 1	115 5	134 1'	117 5	117 0	116 7	114 6	115 1	121 5	121 2	120 5	123 8*
8	138 4	139 3	139 6	156 3+	139 7	139 6	138 6	138 3	137 5	164 6	164 5	164 6	166 3'
9	47 9	43 6	46 1	44 3	49 6	49 0	48 8	38 1	37 8	56 5	50 7	35 5	42 1
10	42 4	37 6	38 1	36 8	34 3	34 5	38 8	33 9	37 1	39 5	40 9	36 6	43 6
11	22 8	22 7	22 1	18 8	21 7	21 8	21 4	22 4	22 0	29 7	31 3	21 5	21 6
12	39 1	39 6	39 3	35 7	38 1	38 9	39 3	40 0	39 6	39 3	39 1	39 1	30 6
13	43 5	43 9	43 6	44 2	43 5	43 5	43 1	44 1	43 7	46 5	48 1	47 6	46 6
14	54 7	54 8	55 0	124 3	55 2	55 1	54 6	54 9	53 8	56 6	85 6	85 5	85 3
15	23 0	22 9	22 9	25 4	23 0	23 0	22 8	22 8	22 8	22 1	31 5	31 7	32 2
16	27 8	27 9	27 9	27 2	28 0	28 0	27 8	28 0	27 5	27 8	26 6	26 6	26 6
17	56 0	56 3	56 2	55 7	56 4	56 3	55 7	56 4	56 0	56 6	50 7	50 7	50 6
18	11 9	11 9	11 9	16 7	11 9	11 9	11 9	11 9	11 9	12 4	16 0	15 9	15 9
19	19 5	22 2	21 2	18 9	13 1	12 5	14 1	23 5	24 1	22 7	22 6	22 8	16 9
20	36 1	36 2	36 2	34 6	36 2	36 2	36 1	36 2	36 2	36 0	35 6	35 6	35 6
21	18 8	18 9	18 9	19 0	18 9	18 9	18 7	18 9	18 8	18 8	18 9	18 9	21 6
22	36 1	36 2	36 2	35 9	36 2	36 2	35 9	36 2	36 0	36 0	35 6	36 3	36 3
23	23 9	24 0	24 0	23 7	24 0	24 0	23 7	24 0	23 9	23 9	24 1	24 1	24 0
24	39 5	39 6	39 6	39 5	39 6	39 6	39 7	39 6	39 5	39 5	39 5	39 5	39 5
25	28 0	28 0	28 0	28 0	28 0	28 0	28 0	28 6	28 0	28 0	28 0	28 0	28 0
26	22.5	22 6-	22.5-	22 6-	22 6-	22 6-	22 5-	22 6-	22 6-	22 6-	22 6-	22.5-	22 5-
27	22 5	22 8-	22 7-	22 8-	22 8-	22 8-	22 8-	22 8-	22 8-	22 8-	22 8-	22 8-	22 8-
1'									49.5"				
2'									50 8"				

Table 1 : ^{13}C NMR chemical shifts

δ_C (50 MHz, standard Me_4Si) * (or +, -, ', ") interchangeable assignment Solvent $CDCl_3$ (77 02)
The assignments were based upon (1) shielding data, (2) by comparison with the spectra of closely related ecdysteroids[a-c] and steroids[d]

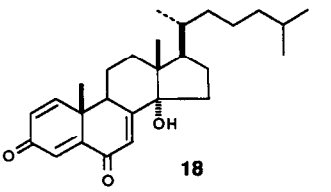
References (a) W B Smith, *Org Magn Reson*, 1977, *9*, 644, (b) T Haag et al, *J Labelled Compd Radiopharm*, 1985, 22, 547
(c) See ref 18 and ref 21, (d) J W Blunt et al, *Org Magn Reson*, 1977, *9*, 439

Hz, 1H, H-2_{ax}) The dehydrobromination was performed in refluxing DMF with LiBr and $LiHCO_3$ and gave the enone **5** (80% yield)

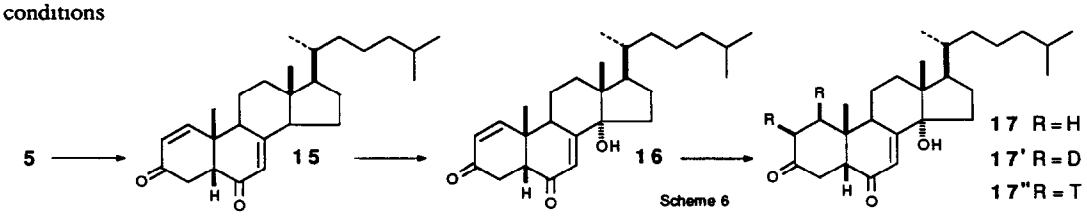


Scheme 5

Oxidation of the allylic position C-6 in compound **5** was carried out in dry methylene chloride using freshly prepared[17] CrO_3/(pyridine)$_2$ complex (Collins reagent), and the dienone **15** was obtained with a moderate yield of 30% (Scheme 6) Oxidation in C-14α must be performed under strong conditions, because the A/B cis ring junction introduces a substantial steric hindrance of the α face of the steroid Treatment of the dienone **15** in refluxing dioxan with 10 equivalents of selenium dioxide[18] afforded in a few minutes compound **16** with a 75% yield. By carefully monitoring the reaction by TLC, the formation of 14α-hydroxy-cholesta-1,4,7-triene-3,6-dione (**18**, by-product) could be minimized[9,19]



Selective reduction of the Δ^1 double bond was performed with palladium on charcoal and yielded the expected compound **17** quantitatively The hindered Δ^7 double bond has never been reduced in our experimental conditions



Scheme 6

Together with the mass spectrometric analysis, ^{1}H NMR spectrometry clearly demonstrated the reduction of the Δ^1 double bond (disappearance of the signals at δ = 5 95 ppm, d, J = 10 0 Hz, 1H, H-2 and δ = 6 83 ppm, d, J= 10 0 Hz, 1H, H-1)

In order to analyse the positions and the number of the hydrogen atoms introduced, we carried out a deuteriation under the conditions which we anticipated to be used in tritiation

^{2}H NMR analysis indicated the introduction of two deuterium atoms (coupling constants of ^{2}H at C-1 and at C-2, δ = 1 52 ppm, m, $w_{1/2}$ = 12 3 Hz, ^{2}H-1 and δ = 2 33 ppm, m, $w_{1/2}$ = 14 2 Hz, ^{2}H-2). The analysis by mass spectrometry of the reaction products gave the following data D_0 5%, D_1 48%, D_2 47%, D_3 0%

To determine the precise configuration of the deuteriums introduced in positions C-1 and C-2, the deuteriated compound **17'** has been reduced using sodium borohydride in methanol to give exclusively the 3-α alcohol[20] (**19**) Under these conditions, the coupling constants of the proton at position C-3β (td, $J_{H3ax-H2eq}$ and $J_{H3ax-H4ax}$ = 11 8 Hz, $J_{H3ax-H4eq}$ = 3 54 Hz, $J_{Deq-H3ax}$ = very weak) were consistent with the incorporation of one deuterium at position C-2β. As the palladium on charcoal is well known to perform cis additions, the second deuterium should be in position C-1β

Tritiation was performed at the Commissariat à l'Energie Atomique (Saclay, France) The specific activity of 14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17"**) was 1 74 TBq/mmole (47 Ci/mmol).

CONVERSION STUDIES OF TRITIATED 14α-HYDROXY-5β-CHOLEST-7-ENE-3,6-DIONE (17")

Prothoracic glands were excised from five larvae of *Locusta* of the mid fifth instar (period of intense ecdysone (**1**) synthesis *in vivo*) and incubated in modified Landureau's medium[21] in the presence of 0 2 μM of the newly-synthesized radiolabelled 5β-diketol **17"** After 16h the medium was injected into a reversed phase HPLC and eluted by a methanol/water gradient (fig 1) In this system, ecdysone and 3-dehydroecdysone are known to co-elute[22] The radioactivity which clearly co-eluted with reference ecdysone was rechromatographed in a system with higher resolution (fig 2) to separate the presumed ecdysone and 3-dehydroecdysone The two molecules are in similar proportions The identity of putative ecdysone was ascertained by co-acetylation with unlabelled ecdysone (data not shown) The identity of putative 3-dehydroecdysone (fractions 30 min - 32 min) was ascertained by a biological approach, as there is not an easy chemical reaction to relate labeled 3-dehydroecdysone to ecdysone But incubation of the putative 3-dehydroecdysone with hemolymph clearly gave some ecdysone (fig 3) These results established the identity of radioactive ecdysone and 3-dehydroecdysone as conversion products of the 5β-diketol **17"** Several experiments were run with regular efficient conversion rates In some cases, up to 80% of the labeled 5β-diketol **17"** incubated was converted into a mixture of ecdysone and 3-dehydroecdysone in similar proportions.

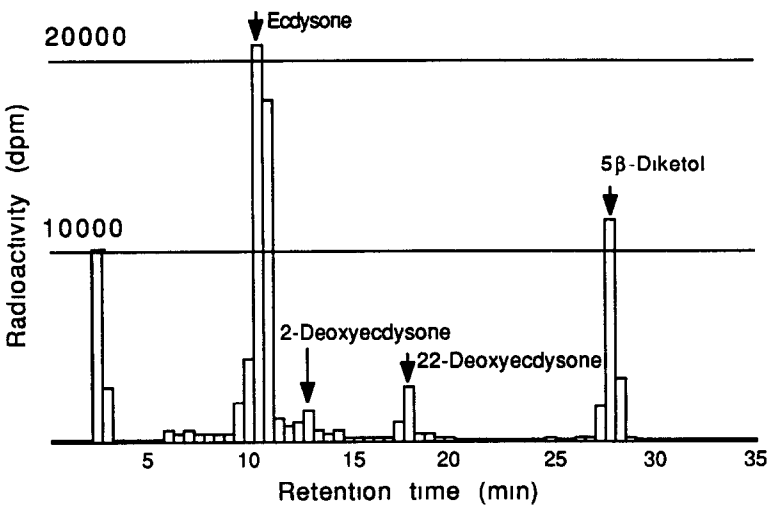


Figure 1 Conversion of tritiated 14α-hydroxy-5β-cholest-7-en-3,6-dione (5β-diketol) by larval prothoracic glands of *Locusta migratoria* Five pairs of prothoracic glands were excised from 5-day old fifth instar larvae, incubated for 22h with 0 2 μM tritiated 5β-diketol after which the radioactive molecules were extracted with methanol and separated on a C-18 reverse phase HPLC column, elution with a gradient from 10% to 100% methanol in water over 30 min Columns radioactivity measurements of aliquots of each fraction Arrows position of reference molecules detected by U V absorbance

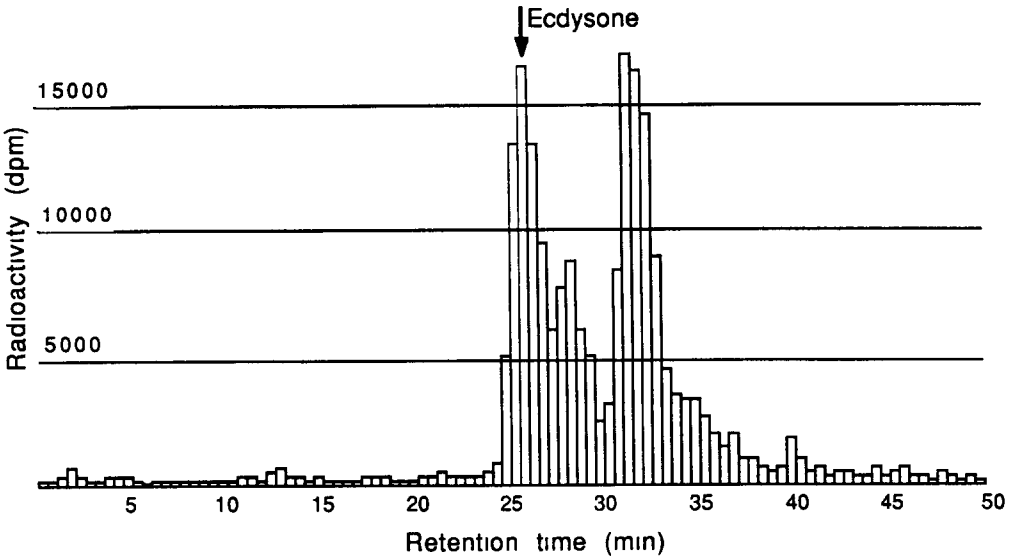


Figure 2 Radiochromatogram of the compound which comigrate with ecdysone, injected together with reference molecule on a C-18 reverse phase HPLC column, elution with a gradient from 15% to 20% acetonitrile in water over 20 min Columns radioactivity measurements of each fraction Arrow position of reference ecdysone detected by U V absorbance

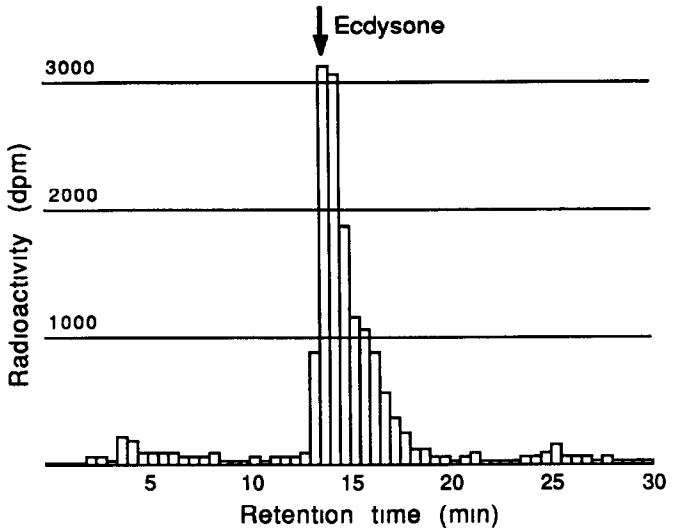


Figure 3 Radiochromatogram of the putative 3-dehydroecdysone after incubation with hemolymph, injected together with reference ecdysone on a C-18 reverse phase HPLC column, isocratic elution with a 20% acetonitrile in water as solvent system Columns radioactivity measurements of each fraction Arrow position of reference ecdysone by U V absorbance.

CONCLUSION

Our initial approach to 14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17**) involved the synthesis of **5** as an intermediate However, the published procedure did not give the desired molecule and it was necessary to synthesize **5** by a different method As a result, our modified synthetic route is novel and also shorter than the original pathway The tritiated molecule **17*** was very efficiently converted to ecdysone (**1**) and 3-dehydroecdysone (**2**) This labelled molecule appears to be a potent precursor in ecdysteroid biosynthesis This first result should now allow investigations into the role of 3-dehydrocompounds in ecdysteroid biosynthesis

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EXPERIMENTAL

Melting points were measured on a Reichert hot stage microscope and are uncorrected $[\alpha]_D$ were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$ IR spectra were recorded in KBr on a Perkin-Elmer 881 infrared spectrophotometer UV spectra were measured on a Kontron-Uvikon 810 UV-vis spectrophotometer NMR spectra were recorded on a Bruker SY (200 MHz) apparatus with $CHCl_3$ (δ = 7 26 ppm) as internal standard for ^1H NMR, $CDCl_3$ (δ = 77 02 ppm) as internal standard for ^{13}C NMR and $CHCl_3$ (δ = 7 26 ppm) as internal standard for ^2H NMR The chemical shifts are reported in ppm downfield from TMS (*, $^+$ = interchangeable assignement) MS were measured on a LKB 9000 S apparatus by direct introduction, or coupled to a GC DB5 column (J W) , an ionization potential of 70 eV was used Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS TLC were run on pre-coated plates of silica gel 60 F 254 (Merck), dipped in a solution of vanillin (1 g) in $EtOH/H_2SO_4$ (95/5, 1 l) and heated on a hot plate to reveal the compounds Medium pressure chromatography (P = 0 5 - 1 1 bar) was conducted on silica gel (40 - 63 mm, Merck) columns Radioactivity has been determined with a Kontron Betamatic V counter (equipped with external standards) All solvents were freshly distilled before use Air- or moisture- sensitive reactions were conducted in flame-dried glassware and under an inert atmosphere

FIRST PATHWAY

Cholesta-4,7-dien-3-one (7)

A toluene solution (400 ml, freshly distilled) containing 7-dehydrocholesterol (**6**) (20 0 g, 52 0 mmol), cyclohexanone (60 ml, 0 58 mol, freshly distilled) and 10 0 g of molecular sieve 0 4 nm was stirred at room temperature for 2 hours After the rapid addition of 6 0 g (29 3 mmol) of aluminium isopropoxide, the orange-red reaction solution was stirred and boiled under reflux for 30 minutes The solution was cooled to room temperature, washed successively with cold (5°C) 2N hydrochloric acid, cold water, cold

aq sat. sodium hydrogen-carbonate and cold brine, dried with sodium sulfate and filtered The toluene solution was concentrated to dryness under reduced pressure and the residue was chromatographed on silica gel Elution with hexane/ethyl acetate 95/5 gave cholesta-4,7-dien-3-one (7), 17 0 g (85%)

7 **Mp** 87-89°C (lit.[7] mp 87-89°C) , $[\alpha]_D$23 + 33 (c = 1 0) , **UV** λ_{max} (ethanol) 238 nm (ε = 15500) , **IR** ν (cm^{-1}) 1690 , 1630 , 1470 , **^{1}H NMR** 200 MHz ($CDCl_3$) δ 0 59 (s, 3H, H-18) , 0 86$^+$ (d, J = 6 61 Hz, 3H, H-26) , 0 87$^+$ (d, J = 6 59 Hz, 3H, H-27), 0 93 (d, J = 6 26 Hz, 3H, H-21) , 1 17 (s, 3H, H-19) , 5 18 (m, $w_{1/2}$ = 9 0 Hz, 1H, H-7) , 5 79 (d, J = 1 72 Hz, 1H, H-4) , **SM** m/z 382 (100 0) (M^+, $C_{27}H_{42}O$) , 367 (9 0) , 339 (10 8) , 338 (37 9) , 259 (20 6) , 247 (11 6) , 227 (10 5) , 136 (20 7) , **Microanalysis** calc for $C_{27}H_{42}O$ (382 3578) C 84 81 , H 11 07 , found C 84 78 , H 11 18 , **^{13}C NMR** in table 1

Cholesta-4,6,8(14)-trien-3-one (8)

First synthesis

A dioxane solution (10 ml) containing cholesta-4,7-dien-3-one (7) (100 0 mg, 0 26 mmol) and DDQ (2,3-dichloro-5,6-dicyano-benzoquinone, 123 9 mg, 0 55 mmol, 2 1 eq) was refluxed during 4 hours After filtration of the hydroquinone, the solution was washed with aq 1% sodium hydroxyde solution and brine, dried with sodium sulfate and filtered. The solution was concentrated to dryness under reduced pressure Cristallisation from acetone gave cholesta-4,6,8(14)-trien-3-one (8), 55 0 mg (55%)

8 **Mp** 61-62°C (lit.[12] mp 61-63°C) , $[\alpha]_D^{20}$ + 635 (c = 1 2) , **UV** λ_{max} (CH_3CN) 240 nm (ε = 4500), 282 nm (ε = 8000), 348 nm (ε = 25500) , **IR** ν (cm^{-1}) 2946 , 2872 , 1662 , 1584 , 1266 , 1220 , 1196 , **^{1}H NMR** 200 MHz ($CDCl_3$) δ 0 87 (d, J = 6 62 Hz, 6H, H-26,27) , 0 96 (d, J = 6.51 Hz, 3H, H-21) , 0 95 (s, 3H, H-19) , 0 99 (s, 3H, H-18) , 5 73 (s, 1H, H-4) , 6 03* (d, J = 9 29 Hz, 1H, H-7) , 6 62* (d, J = 9 29 Hz, 1H, H-6) , **SM** m/z 380 (68 6) (M^+, $C_{27}H_{40}O$) , 365 (17 6) , 268 (100 0) , 214 (25 9) , **Microanalysis** calc for $C_{27}H_{40}O$ (380 6120) C 85 20 , H 10 59 , found C 85 22 , H 10 51 , **^{13}C NMR** in table 1

Second synthesis

To a tetrahydrofuran solution (10 ml) containing cholesta-4,7-dien-3-one (7) (100 0 mg, 0 26 mmol) was added dropwise at 0°C tri-N-methylanilinium perbromide (108 0 mg, 0 28 mmol, 1 1 eq) in tetrahydrofuran (8 ml) After addition (5 minutes), the mixture was stirred 15 minutes at 0°C and at room temperature for a further 15 minutes The mixture was then poured into water and the product isolated with ether The organic layer was washed with water, brine dried with sodium sulfate and evaporated to dryness The residue was chromatographed on silica gel Elution with hexane/ethyl acetate 95/5 gave cholesta-4,6,8(14)-trien-3-one (8), 63 1 mg (63%)

SECOND PATHWAY

5α-Cholest-7-en-3β-ol (9)

7-dehydrocholesterol (6) (10 0 g, 26 0 mmol) was dissolved in a 1/1 (v/v) mixture of toluene and ethyl acetate Hydrogenation was conducted over palladium (5%) on activated charcoal (1 5 g) at room temperature and atmospheric pressure during 2 hours The solution was filtered on celite and evaporated to dryness to give 5α-cholest-7-en-3β-ol (9), 9 85 g (98%)

9 **^{1}H NMR** 200 MHz ($CDCl_3$) δ 0 53 (s, 3H, H-18) , 0 79 (s, 3H, H-19) , 0 86 (d, J = 6 56 Hz, 6H, H-26,27) , 0 94 (d, J = 5 97 Hz, 3H, H-21) , 3 64 (m, $w_{1/2}$ = 25 0 Hz, 1H, H-3) , 5 16 (m, $w_{1/2}$ = 6 7 Hz, 1H, H-7) , **SM** m/z 386 (100 0) (M^+, $C_{27}H_{46}O$) , 371 (23.6) , 273 (14 4) , 255 (47 1) , 231 (14 9) , 228 (14 4) , 213 (15 5) , **Microanalysis** calc for $C_{27}H_{46}O$ (386 6594) C 83 87 , H 11 99 , found C 83 82 , H 11 75 , **^{13}C NMR** in table 1

5α-Cholest-7-en-3-one (10)

To a solution of 5α-cholest-7-en-3β-ol (9) (7 5 g, 19 4 mmol) in dry methylene chloride (200 ml) was added 9 0 g of pyridinium-chlorochromate absorbed on alumina After stirring for 12 hours, the solid is filtered on alumina and washed with ethyl acetate The combined filtrates were evaporated to dryness to afford pure 5α-cholest-7-en-3-one (10), 7 1 g (95%)

10 Mp 144-146°C (lit.[12] mp 144-146°C), $[\alpha]_D^{20}$ + 25 (c = 3 9), **IR** ν (cm^{-1}) 1725, 1665, ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 56 (s, 3H, H-18), 0 87 (d, J = 6 53 Hz, 6H, H-26,27), 0 93 (d, J = 6 31 Hz, 3H, H-21), 1 01 (s, 3H, H-19), 5 19 (b, $w_{1/2}$ = 9 0 Hz, 1H, H-7), **SM** m/z 384 (100 0) (M^+, $C_{27}H_{44}O$), 369 (31 1), 272 (15 3), 271 (65 5), 245 (15.2), 244 (16 5), 229 (44 3), **Microanalysis** calc for $C_{27}H_{44}O$ (384 6436) C 84 31, H 11 53, found C 83 12, H 11 90, ^{13}**C NMR** in table 1

2α,4α-Dibromo-5α-cholest-7-en-3-one (11)

A solution of 5α-cholest-7-en-3-one (10) (1 0 g, 2 6 mmol) in tetrahydrofuran (20 ml) containing tri-N-methylanilinium perbromide (2 0 g, 5 3 mmol, 2 1 eq) was stirred during 15 minutes at 0°C and at room temperature for a further 15 minutes The mixture was then poured into water and the product isolated with ether The organic layer was washed with water, brine dried with sodium sulfate and evaporated to dryness to yield 2α,4α-dibromo-5α-cholest-7-en-3-one (11), 630 mg (64%), wich was normally used directly for the next reaction (dielimination) Recristallization from methanol/ether of an aliquot gave 2α,4α-dibromo-5α-cholest-7-en-3-one as white needles

11 Mp 195-197°C (lit.[12] mp 192-195°C), $[\alpha]_D^{20}$ - 20 (c = 0 9), **UV** λ_{max} (CH_3CN) 197 nm (ε = 13200), **IR** ν (cm^{-1}) 2962, 2844, 1741, 1455, 1388, 1163, ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 57 (s, 3H, H-18), 0 87 (d, J = 6 49 Hz, 6H, H-26,27), 0 91 (d, J = 6 14 Hz, 3H, H-21), 1 20 (s, 3H, H-19), 2 55 (b, $w_{1/2}$ = 26 3 Hz, 1H, H-6_{eq}), 2 75 (dd, J = 13 16 et 5 26 Hz, 1H, H-1_{eq}), 4 67 (d, J = 13 16 Hz, 1H, H-4_{ax}), 4 87 (dd, J = 14 21 et 5 78 Hz, 1H, H-2_{ax}), 5 25 (bd, $w_{1/2}$ = 11 6 Hz, 1H, H-7), **SM** m/z 544 (53 1), 543 (31 0), 542 (100 0) (M^+, $C_{27}H_{42}OBr_2$), 462 (39 5), 431 (47 1), 429 (93 1), 427 (49 4), 387 (41 1), 381 (40 6), 323 (57 5), **Microanalysis** calc for $C_{27}H_{42}OBr_2$ (542 4358) C 59 78, H 7 80, found C 59 60, H 7 60, ^{13}**C NMR** in table 1

Cholesta-1,4,7-trien-3-one (4)

A solution of the unpurified dibromide 11 (3 0 g, 5 5 mmol) in dimethylformamide (20 ml) containing lithium carbonate (5 0 g) and lithium bromide (2 5 g) was refluxed during 30 minutes The solution was cooled to room temperature, washed with water and brine, dried with sodium sulfate and evaporated to dryness The residue was chromatographed on silica gel Elution with hexane/ethyl acetate 95/5 gave cholesta-1,4,7-trien-3-one (4), 2 7 g (90%)

4 Mp 130-132 °C (lit.[12] mp 130-132°C), $[\alpha]_D^{20}$ - 15 (c = 2 8), **UV** λ_{max} (CH_3CN) 243 nm (ε = 14800), **IR** ν (cm^{-1}) 1650, 1625, 1600, ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 63 (s, 3H, H-18), 0 87 (d, J = 6 48 Hz, 6H, H-26,27), 0 94 (d, J = 6 28 Hz, 3H, H-21), 1 24 (s, 3H, H-19), 2 81 (d, J = 18 80 Hz, 1H, H-6), 3 17 (d, J = 18 80 Hz, 1H, H-6), 5.25 (m, $w_{1/2}$ = 10 0 Hz, 1H, H-7), 6 13 (m, $w_{1/2}$ = 5 0 Hz, 1H, H-4), 6.26 (dd, J = 9 99 Hz, 1H, H-2), 7 09 (d, J = 10 05 Hz, 1H, H-1), **SM** m/z 380 (100 0) (M^+, $C_{27}H_{40}O$), 365 (28 0), 268 (31 2), 172 (48 9), 157 (59 3), 133 (64 8), 121 (59 0), 105 (42 0), **Microanalysis** calc for $C_{27}H_{40}O$ (380 6120) C 85 20, H 10 59, found C 84 93, H 10 78, ^{13}**C NMR** in table 1

Cholesta-4,7-dien-3-one (7)

Cholesta-1,4,7-trien-3-one (4) (200 0 mg, 0 53 mmol) was dissolved in ethanol (10 ml) After addition of 20 ml of quinoline, the hydrogenation was conducted over palladium (5%) on calcium carbonate (20 mg) at room temperature during 16 minutes (1 Atm pressure) The solution was immediately filtered and evaporated to dryness The residue was chromatographed on

silica gel Elution with hexane/ethyl acetate 97/3 gave a mixture of 5β-cholest-7-en-3-one (12), 35 0 mg (17%), cholesta-4,7-dien-3-one (7), 120 0 mg (60%) and starting material 4, 20 0 mg (10%)

The previous procedure, without quinoline or using Palladium (5%) on activated charcoal, gave the same products, but with different ratio

5β-Cholest-7-en-3-one (12)

Cholesta-1,4,7-trien-3-one (4) (200 0 mg, 0 53 mmol) was dissolved in ethanol (10 ml) After addition of 20 ml of quinoline, the hydrogenation was conducted over palladium (5%) on calcium carbonate (20 mg) at room temperature during 30 minutes The solution was immediately filtered and evaporated to dryness The residue was chromatographed on silica gel Elution with hexane/ethyl acetate 97/3 gave 5β-cholest-7-en-3-one (12), 179 0 mg (90%)

The previous procedure, without quinoline or using Palladium (5%) on activated charcoal, gave the same product

12 **Mp** 87-88°C (lit.[23] mp 87-88°C) , $[\alpha]_D^{23}$ + 65 (c = 0 57) , **IR** ν (cm^{-1}) 1725 , 1665 , ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 57 (s, 3H, H-18) , 0 87 (d, J = 6 61 Hz, 6H, H-26,27) , 0 93 (d, J = 6 24 Hz, 3H, H-21) , 0 99 (s, 3H, H-19) , 5 12 (m, $w_{1/2}$ = 10 0 Hz, 1H, H-7) , **SM** m/z 384 (92 4) (M^+, $C_{27}H_{44}O$) , 369 (20 4) , 352 (30 0) , 351 (100 0) , 334 (30 3) , 333 (49 8) , 119 (32 6) , 105 (68 0) , **Microanalysis** calc for $C_{27}H_{44}O$ (384 6436) C 84 31 , H 11 53 , found C 83 99 , H 11 62 , ^{13}**C NMR** in table 1

THIRD PATHWAY

5β-Cholest-7-en-3-one (12)

A solution of cholesta-4,7-dien-3-one (7) (2 0 g, 5 2 mmol), 60 ml of ethanol, 12 ml of aq 0 1N sodium hydroxide and 200 0 mg of palladium (5%) on activated charcoal (150 mg) was shaken with hydrogen at room temperature and atmospheric pressure for 2 hours. The catalyst was removed by filtration The solution was washed with water, brine and then dried with sodium sulfate and evaporated to dryness The residue was chromatographed on silica gel Elution with hexane/ethyl acetate 95/5 gave 1 4 g of 5β-cholest-7-en-3-one (12), (72%) and 150 0 mg of 5α-cholest-7-en-3-one (10), (7%)

5β-cholesta-1,7-dien-3-one (5)

First step

A solution of 5β-cholest-7-en-3-one (12) (435 0 mg, 1 1mmol) in tetrahydrofuran (20 ml) containing tri-N-methylanilinium perbromide (468 0 mg, 1 24 mmol, 1 1 eq) was stirred during 15 minutes at 0°C and at room temperature for a further 15 minutes The mixture was then poured into water and the product isolated with ether The organic layer was washed with water, brine, dried with sodium sulfate and evaporated to dryness to yield 2α-bromo-5β-cholest-7-en-3-one (13), 350 mg (80%) (which was normally used directly for the next reaction) Cristallisation from methanol/ether of an aliquot gave 2α-bromo-3,3-dimethoxy-5β-cholest-7-ene (14) in white needles

14 **Mp** 124-126°C , $[\alpha]_D^{23}$ + 22 (c = 0 89) , **IR** ν (cm^{-1}) 2960 , 2918 , 2875 , 1458 , 1378 , 1119 , 1098, 1053 , ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 53 (s, 3H, H-18) , 0 87 (d, J = 6 62 Hz, 6H, H-26,27) , 0 90 (s, 3H, H-19) , 0 92 (d, J = 6 14 Hz, 3H, H-21) , 2 22 (dd, J = 14 50 et 5 00 Hz, 1H, H-1_{eq}) , 2 41 (b, $w_{1/2}$ = 20 00 Hz, 1H, H-6_{eq}) , 3 34 (s, 3H, H-1') , 3 40 (s, 3H, H-1") , 4 34 (dd, J = 12 97 et 4 46 Hz, 1H, H-2_{ax}) , 5 10 (bd, $w_{1/2}$ = 10 0 Hz, 1H, H-7) , **SM** m/z 510 (4 1) , 508 (3 0) (M^+, $C_{29}H_{49}O_2Br$) , 478 (22 3) , 476 (21 9) , 398 (32 6) , 397 (100 0) , 366 (28 3) , 365 (85 1) , 314 (21 2) , 313 (57 9) , 279 (14 1) , 256 (30 6) , 242 (15 9) , 228 (19 5) , **Microanalysis** calc for $C_{29}H_{49}O_2Br$ (509 6081) C 68 35 , H 9 69 , found C 68 19 , H 9 40 , ^{13}**C NMR** in table 1

Second step

A solution of the unpurified bromide **13** (350 0 mg, 0 76 mmol) in dimethylformamide (15 ml) containing lithium carbonate (1 4 g) and lithium bromide (750 0 mg) was refluxed during 30 minutes. The solution was cooled to room temperature, washed with water and brine, dried with sodium sulfate and evaporated to dryness The residue was chromatographed on silica gel Elution with hexane/ethyl acetate 95/5 gave a mixture of 5β-cholesta-1,7-dien-3-one (**5**), 283 1 mg (80 1%), cholesta-4,7-dien-3-one (**7**), 27 5 mg (7 8%) and cholesta-1,4,7-trien-3-one (**4**), 11 7 mg (3 3%)

5 **Mp** 93-95 °C , $[\alpha]_D^{23}$ + 41 (c = 1 0) , **UV** λ_{max} (CH_3CN) 196 nm (ε = 14100), 230 nm (ε = 11100) , **IR** ν (cm^{-1}) 2959 , 2934 , 2875 , 1667 ; 1466 , 1442 , 1412 , 1372 , ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 57 (s, 3H, H-18) , 0 86 (d, J = 6 54 Hz, 6H, H-26,27) , 0 92 (d, J = 6 29 Hz, 3H, H-21) , 1 26 (s, 3H, H-19) , 5.15 (b, $w_{1/2}$ = 10 0 Hz, 1H, H-7) , 5 88 (d, J = 10 00 Hz, 1H, H-2) , 6 98 (d, J = 10.03 Hz, 1H, H-1) , **SM** m/z 382 (87 4) (M^+, $C_{27}H_{42}O$) , 275 (23 3) , 274 (100.0) , 269 (16 1) , 161 (22.5) , 109 (25 4) , **Microanalysis** calc for $C_{27}H_{42}O$ (382 6278) C 84 75 , H 11 06 , found C 84 92 , H 11 18 , ^{13}**C NMR** in table 1

5β-Cholesta-1,7-diene-3,6-dione (15)

In 10 ml of methylene chloride at room temperature and under a nitrogen atmosphere, 100 0 mg (0 26 mmol) of 5β-cholesta-1,7-dien-3-one (**5**) was dissolved To the stirred solution was added 700 mg (2 6 mmol) of CrO_3-(pyridine)$_2$ complex (Collins reagent, freshly prepared) The mixture immediately began turning brown and depositing a tarry precipitate on the sides and bottom of the flask. After 15 hours of stirring at room temperature, an additional 700 mg (2.6 mmol) of CrO_3-(pyridine)$_2$ complex was added to the reaction mixture 4 hours later, thin layer analysis of the solution showed the presence of a trace of starting material (longer oxidation periods did not eliminate this product) The reaction mixture was poured from the flask. The precipitate remaining in the flask was rinsed with small portions of ether (little improvement in yield was found when the tarry precipitate was dissolved in aq. sat. sodium hydrogen-carbonate and then extracted with ether) The organic layer was washed several times with aq sat. sodium hydrogen-carbonate, 5% hydrochloric acid, dried with sodium sulfate and filtered. The solvents were evaporated to dryness and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 95/5 gave 5β-cholesta-1,7-diene-3,6-dione (**15**), 27 0 mg (27%)

15 **Mp** 122-124°C , $[\alpha]_D^{23}$ + 33 (c = 1 02) , **UV** λ_{max} (CH_3CN) · 194 nm (ε = 13900), 225 nm (ε = 18700) , **IR** ν (cm^{-1}) 2927 , 2867 , 1654 , 1455 , 1380 , 1262 , ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 62 (s, 3H, H-18) , 0.87 (d, J = 6 55 Hz, 6H, H-26,27) , 0 93 (d, J = 5.88 Hz, 3H, H-21) , 1 26 (s, 3H, H-19) , 5 77 (bs, $w_{1/2}$ = 5 0 Hz, 1H, H-7) , 5 95 (d, J = 10.17 Hz, 1H, H-2) , 6 79 (d, J = 10 18 Hz, 1H, H-1) , **SM** m/z 396 (85.5) (M^+, $C_{27}H_{42}O_2$) ; 381 (19 0) , 378 (23 1) , 288 (22 1) , 284 (23 0) , 183 (100.0) , 185 (24.5) , **Microanalysis** calc for $C_{27}H_{40}O_2$ (396 6110) C 81 77 , H 10 16 , found C 81 95 , H 10 03 , ^{13}**C NMR** in table 1.

14α-Hydroxy-5β-cholesta-1,7-diene-3,6-dione (16)

To a solution of 250 0 mg (0 63 mmol) of 5β-cholesta-1,7-diene-3,6-dione (**15**) in freshly distilled dioxan (20 ml), was added 700 mg (6.31 mmol, 10 eq) of finely powdered SeO_2 and the mixture was refluxed during 15 minutes. The solution was then cooled to 0°C and allowed to warm to room temperature After filtration of the solution on celite, the solvent was removed. The residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 92/8 gave 14α-hydroxy-5β-cholesta-1,7-diene-3,6-dione (**16**), 197 6 mg (76%) and 14α-hydroxy-cholesta-1,4,7-triene-3,6-dione (**18**), 25 0 mg (10%)

16 **Mp** 179-181°C , $[\alpha]_D^{23}$ + 73 (c = 0.75) , **UV** λ_{max} (CH_3CN) : 194 nm (ε = 10700), 224 nm (ε = 19400) , **IR** ν (cm^{-1}) 3457 , 2954 , 2908 ; 1662 ; 1456 , 1379 , ^{1}**H NMR** 200 MHz ($CDCl_3$) δ . 0 71 (s, 3H, H-18) , 0 87 (d, J = 6 52 Hz, 6H, H-26,27) , 0.93 (d, J = 6 22 Hz, 3H, H-21) , 1.27 (s, 3H, H-19) , 3 14 (bt, $w_{1/2}$ = 20.0 Hz, 1H, H-9) , 5 92 (d, J = 2 10 Hz,

1H, H-7), 5 95 (d, J = 10 03 Hz, 1H, H-2), 6 82 (d, J = 10 05 Hz, 1H, H-1), **SM** m/z 412 (34 1) (M^+, $C_{27}H_{40}O_3$), 394 (51 0), 277 (27 3), 276 (100 0), 229 (42 9), 109 (34 4), **Microanalysis** calc for $C_{27}H_{40}O_3$ (412 6100) C 78 59, H 9 77, found C 78 78, H 9.50, ^{13}**C NMR** in table 1

18 Mp 95-97°C, ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 78 (s, 3H, H-18), 0 88 (d, J = 6 51 Hz, 6H, H-26,27), 0 94 (d, J = 6 17 Hz, 3H, H-21), 1 34 (s, 3H, H-19), 3 06 (bt, $w_{1/2}$ = 20 0 Hz, 1H, H-9), 6 17 (bs, $w_{1/2}$ = 5 0 Hz, 1H, H-7), 6 33 (d, J = 10 20 Hz, 1H, H-1), 6 68 (bs, $w_{1/2}$ = 5 0 Hz, 1H, H-4), 7 06 (d, J = 9 82 Hz, 1H, H-2), **SM** m/z 410 (66 8) (M^+, $C_{27}H_{38}O_3$), 382 (38 4), 228 (82 7), 227 (72 4), 200 (34 1), 135 (97 0), **Microanalysis** calc for $C_{27}H_{38}O_3$ (410 5942) C 78 98, H 9 33, found C 79 03, H 9 40, ^{13}**C NMR** in table 1

14α-Hydroxy-5β-cholest-7-ene-3,6-dione (17)

The compound **16** (100 0 mg, 0 24 mmol) was dissolved in ethyl acetate. Hydrogenation was conducted over palladium (5%) on activated charcoal (20 0 mg) at room temperature and atmospheric pressure during 1 hour The solution was filtered and evaporated to dryness to give 14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17**), 98 mg (98%)

17 Mp 172-174°C, $[\alpha]_D^{23}$ + 75 (c = 0 92), **UV** λ_{max} (CH_3CN) 194 nm (ε = 11800), 243 nm (ε = 14700), **IR** ν (cm^{-1}) 3502, 2964, 1720, 1643, 1462, 1378, 1318, 1253, ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 71 (s, 3H, H-18), 0 87 (d, J = 6 52 Hz, 6H, H-26,27), 0 93 (d, J = 6 22 Hz, 3H, H-21), 1 09 (s, 3H, H-19), 3 27 (bt, $w_{1/2}$ = 20 0 Hz, 1H, H-9), 5 87 (d, J = 2 18 Hz, 1H, H-7), **SM** m/z 414 (87 3) (M^+, $C_{27}H_{42}O_3$), 386 (67 6), 234 (76 2), 232 (79 7), 231 (100 0), **Microanalysis** calc for $C_{27}H_{42}O_3$ (414 6258) C 78 21, H 10 21, found C 77 90; H 10 14, ^{13}**C NMR** in table 1

[1,2-$^2H_2$]-14α-Hydroxy-5β-cholest-7-ene-3,6-dione (17')

The procedure described above, under deuterium atmosphere (1 Atm pressure, with 7 5 mg of 14α-Hydroxy-5β-cholesta-1,7-diene-3,6-dione (**16**)), gave [1,2-$^2H_2$]-14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17'**) quantitatively (100%)

17' ^{1}**H NMR** 200 MHz ($CDCl_3$) δ = 0 71 (s, 3H, H-18), 0 87 (d, J = 6.52 Hz, 6H, H-26,27), 0 93 (d, J = 6 22 Hz, 3H, H-21), 1 09 (s, 3H, H-19), 3 27 (bt, $w_{1/2}$ = 20 0 Hz, 1H, H-9), 5 87 (d, J = 2 18 Hz, 1H, H-7), ^{2}**H NMR** 61 4 MHz ($CHCl_3$) δ 1 52 (b, $w_{1/2}$ = 10 1 Hz, 1D, D-1), 2 33 (b, $w_{1/2}$ = 12 3 Hz, 1D, D-2), **SM** m/z 417 (17 9), 416 (56 7) (M^+, $C_{27}H_{40}O_3D_2$), 415 (45 5), 389 (17 0), 388 (51 6), 236 (49 1), 235 (61 8), 234 (63 5), 233 (100 0), 232 (62 8), 174 (20 8), 161 (29 1)

[1,2-$^2H_2$]-3α,14α-Dihydroxy-5β-cholest-7-en-6-one (19)

To a solution of [1,2-$^2H_2$]-14α-hydroxy-5β-cholest-7-ene-3,6-dione (**17'**) (2 0 mg, 4 8 mmol) in methanol (1 ml) was added 2 0 mg of sodium borohydride The mixture was stirred 5 minutes, then 1 ml of water was added. Extraction with ether gave pure [1,2-$^2H_2$]-3α,14α-dihydroxy-5β-cholest-7-en-6-one (**19**), 1 5 mg (75 %)

19 ^{1}**H NMR** 200 MHz ($CDCl_3$) δ 0 68 (s, 3H, H-18), 0 87 (d, J = 6 51 Hz, 6H, H-26,27), 0 93 (d, J = 6 45 Hz, 3H, H-21), 1 23 (s, 3H, H-19), 3 18 (bt, $w_{1/2}$ = 20 0 Hz, 1H, H-9), 3 66 (td, J = 10 78 et 3 53 Hz, 1H, H-3), 5 87 (d, J = 2 45 Hz, 1H, H-7), **SM** m/z 418 (21 9) (M^+, $C_{27}H_{42}O_3D_2$), 400 (41 2), 399 (25 0), 390 (45 8), 389 (26.8), 372 (28 7), 235 (26 4), 217 (21 7)

[1,2-$^3H_2$]-14α-Hydroxy-5β-cholest-7-ene-3,6-dione (17")

The procedure described above was carried out under a tritium atmosphere at the CEA (Saclay)

Compound **16** (2 5 mg, 8 0 μmol) was dissolved in ethyl acetate (2 ml) Tritiation (tritium gas 0 67 TBq, 20 Ci) was conducted (via a Toepler pump) over palladium (5%) on activated charcoal (4 0 mg) at room temperature and at 500 mmHg pressure

during 40 mn The solution was filtered and evaporated to dryness The labile tritium was eliminated by dilution in methanol and evaporation, twice The compound 17" (6 9 GBq, 186 mCi) was obtained with a radiochemical purity about 96% by High Performance Liquid Chromatography (HPLC, silica column, hexane/ethyl acetate 77/23) The analytical radio-chromatography was made on an reverse phase column in analytical HPLC (C-18 ODS, methanol/water 90/10)

17" ^{3}H NMR 320 MHz (Bruker C300, ^{1}H decoupled) (DMSO) δ 1 43 (d, J = 1 63 Hz, 1T, T-1) , 2 14 (d, J = 1 63 Hz, 1T, T-2) , SM (Finnigan 4600, DCI/NH_3) m/z 436 (29 6) ([M+NH_3]$^+$, $C_{27}H_{40}O_3T_2$ + NH_3) , 434 (13 5) , 432 (1 9) , Specific activity 1 74 TBq/mmol (47 Ci/mmol)

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