Tetrahedron Vol. 49, No. 12, pp. 2485-2498, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Synthesis of a Tritiated 3-Dehydroecdysteroid Putative Precursor of Ecdysteroid Biosynthesis

Frédéric DOLLE[1], Charles HETRU[2], Bernard ROUSSEAU[3], Frank SOBRIO[3]
Catherine BLAIS[4], René LAFONT[4], Michel DESCAMP[5] & Bang LUU[1]*

[1]Laboratoire de Chimie Organique des Substances Naturelles, associé au CNRS
5 rue Blaise Pascal, 67084 Strasbourg, France.
[2]Laboratoire de Biologie Générale, associé au CNRS
12 rue de l'Université, 67000 Strasbourg, France.
[3]Service des Molécules Marquées, Departement de Biologie Cellulaire et moléculaire
CE de Saclay, 91191 Gif-sur-Yvette, France.
[4]Laboratoire de Biochimie et Physiologie du Développement, associé au CNRS
ENS, 46 rue d'Ulm, 75230 Paris Cedex 05, France.
[5]Laboratoire de Biologie Animale, Université de Lille I
59655 Villeneuve d'Ascq Cedex, France.

(*Received in Belgium* 4 *December* 1992)

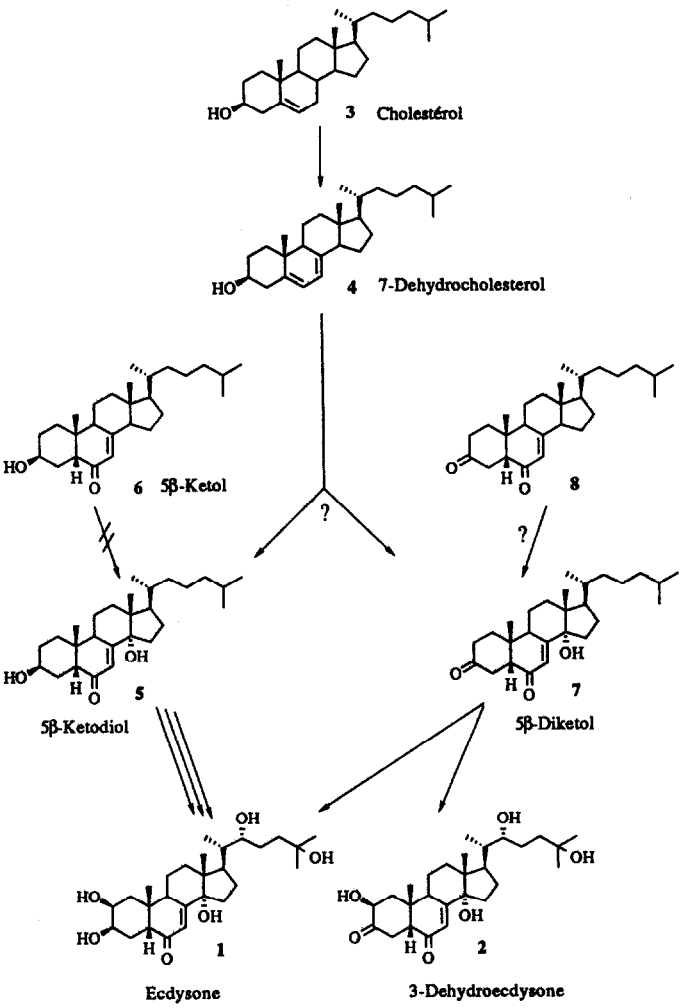
Abstract - We have synthesized a tritiated form of 5β-cholest-7-ene-3,6-dione (5β-diketone) of high specific activity (9.2 TBq/mmol) in ten steps from deoxychenocholic acid, which possesses the desired A/B cis ring junction (5β-H configuration). We have conceived a synthetic pathway which permits labelling by tritiation at the final step and allowing for the introduction of the tritium label into both the side chain and the nucleus. We have examined the ability of insect endocrine glands (prothoracic glands) of *Pieris Brassicae* to use this molecule as a precursor of 3-dehydroecdysone and of ecdysone biosynthesis. No conversion can be detected. In the myriapod *Lithobius forficatus* only reduction of the 3-ketone can be observed. It seems that the 5β-diketone is not an intermediate in the ecdysone biosynthetic pathway.

Introduction

Essential events in the development and reproduction of arthropods are controlled by the steroid hormone family : the ecdysteroids (Scheme 1). Ecdysone (**1**), the parent molecule of this family, is produced during postembryonic development in specialized endocrine glands. Recently, it has been reported that in some insect and crustacean species, the endocrine glands secrete 3-dehydroecdysone (**2**)[1,2] in addition to ecdysone.

It is well documented that cholesterol (**3**) and 7-dehydrocholesterol (**4**) are precusors of ecdysone (**1**)[3,4]. In spite of a relatively large number of investigations, the biochemical events leading from cholesterol (**3**) to ecdysone (**1**) have not been completely elucidated[4]. The steps of hydroxylation which occur on the side chain at C-25 and C-22 followed by hydroxylation on the A ring at C-2 are now clearly established[4]. However, the intermediates between 7-dehydrocholesterol (**4**) and 3β,14α-dihydroxy-5β-cholest-7-en-6-one (5β-ketodiol **5**, the substrate of the C-25 hydroxylation) are unknown and their structures have only been hypothesized[5]. Based on its presence in organs which produce ecdysone, it has recently been proposed that 3β-hydroxy-5β-cholest-7-en-6-one (5β-ketol, **6**) could be an ecdysone precursor and a substrate for a C-14 hydroxylase[6], but we have been unable to confirm this assumption[7]. Recently, we have shown that 14α-hydroxy-5β-cholest-7-ene-3,6-dione (5β-diketol, **7**) was very efficiently converted into ecdysone and 3-dehydroecdysone in insects[8]. In the past, it has been proposed[9,10] that the beginning of the biosynthetic pathway involved 3-dehydro-compounds.

Thus, we have undertaken the synthesis of 5β-cholest-7-ene-3,6-dione (5β-diketone, **8**) in labelled form and investigated its biological conversion to ecdysone (**1**) and/or 3-dehydroecdysone (**2**).

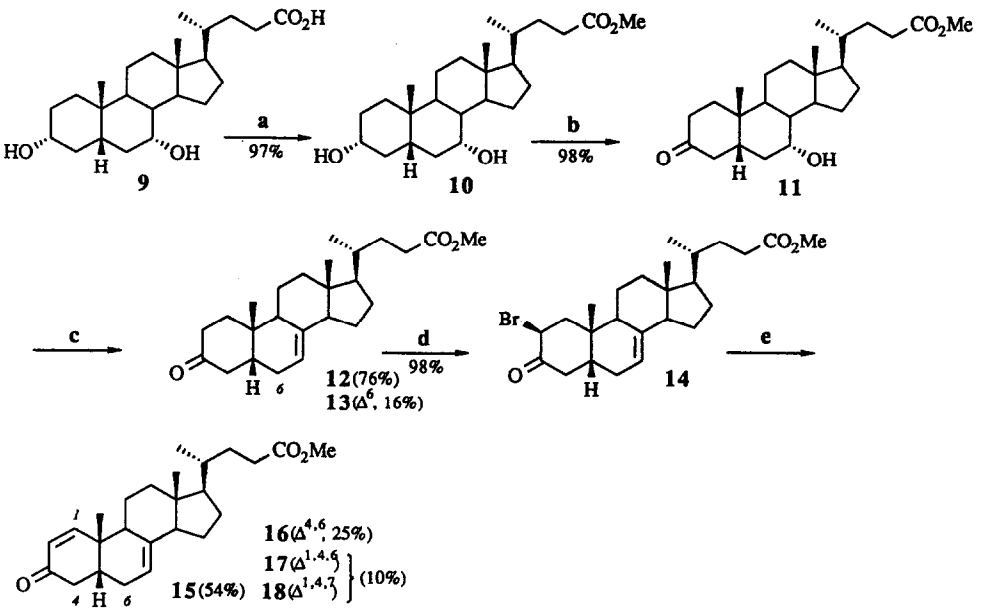


Biosynthetic pathway of ecdysone and 3-dehydroecdysone
Scheme 1

Our chosen target molecule (**8**) possesses an A/B cis ring junction (5β-H configuration) and the synthetic strategy which we have developed in the present paper is to start from a commercial steroid which possesses this desired configuration : deoxychenocholic acid. In this compound the functionality at C-3, C-7 and C-24 can lead to the desired molecule. We have conceived a synthetic pathway which permits labelling by tritiation at the final step. Furthermore, this strategy allows for the introduction of the tritium label into both the side chain and the nucleus to give a high specific activity and thus allows for the detection of metabolites even after side chain cleavage.

Synthesis of [1β,2β,24,25-$^3H_4$]-5β-cholest-7-ene-3,6-dione (8")

The C-24 acid group of the deoxychenocholic (**9**) acid was esterified to produce 24-methyl-deoxychenocholate (**10**) using aqueous concentrated HCl in refluxing methanol. This reaction was very efficient (97% yield) and was more convenient than the diazomethane technique (Scheme 2).



Reagents and conditions: (a) HCl, MeOH, reflux, 15mn; (b) O_2, Pt, AcOEt, 25°C, 100h; (c) $POCl_3$, Py, 25°C, 16h (76%); (d) $(CH_3)_3C_6H_5N^+Br^-.Br_2$ (1.05 eq), THF, 0°C, 15mn, then 25°C, 30mn; (e) LiBr, Li_2CO_3, DMF, reflux, 30mn.

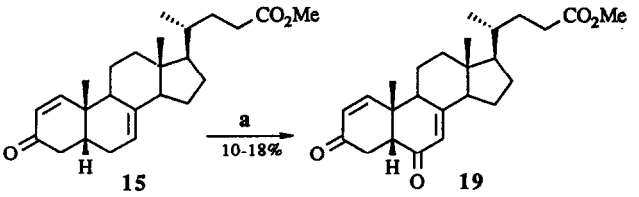
Scheme 2

The regioselective oxidation of the 3α-hydroxyl group was performed using platinum (generated *in situ* from PtO_2 and hydrogen) and oxygen in ethyl acetate[11]. During the preparation of the reagents, it is important to avoid the simultaneous presence of hydrogen, oxygen and platinum (See experimental part). Compound **11** was obtained in very good yield (98%), the hydroxyl group in C-7α being preserved under these experimental conditions.

The hydroxyl group in C-7α was then eliminated at room temperature using $POCl_3$ in pyridine[12]. The compounds **12** and **13** were isolated in yields of 76% (Δ^7) and 16% (Δ^6) respectively.

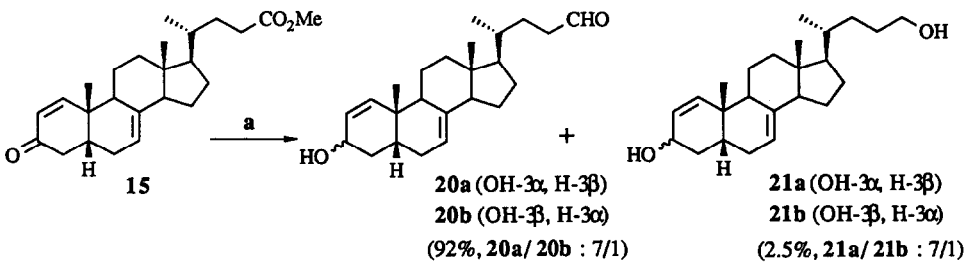
Compound **15** was obtained in two steps[13] : a) regio and stereo selective bromination in α position of the C-3 ketone, carried out at 0°C with tri-N-methylanilinium perbromide in THF, to give the 2-bromo compound **14**. b) elimination of HBr using lithium bromide and lithium carbonate in refluxing DMF, afforded the α,β unsaturated ketone **15** (58% yield from **12**). Other ketones were also isolated and partially characterized [(**16** (25%), mixture of **17** and **18** (10%). Even under various other conditions, we did not succeed in improving the yields of **15**.

The only usual technique to introduce the 6-ketone is allylic oxidation using the complex $CrO_3.Py_2$ (Collins complex)[14], but the yield obtained was low (Scheme 3; in assays with **15** to give **19** the best isolated yield we observed was 18%). Consequently, we have preferred to position this step at the end of the synthesis.



Reagents and conditions: (a) $CrO_3.Py_2$ (32 eq), CH_2Cl_2, 21h, 25°C.
Scheme 3

The conversion of the C-24 methyl ester to the aldehyde (Scheme 4) can be performed by reduction with hydride, but it is necessary to optimise the reaction conditions in order to stop the reduction at the aldehyde level and minimise the formation of alcohol. The reaction was performed by cautious addition, at -78°C, of the diisobutyl-aluminium hydride (1M in hexane) in a solution of the ester **15** in a mixture of *tert*-butyl-methyl ether and dicholoromethane (65/35, v/v). When the temperature was kept below -75°C, the aldehydes **20a** and **20b** were obtained in very high yield (92%), and the diols **21a** and **21b** were present in only 2.5% yield.



Reagents and conditions: (a) DIBAH (2.05 eq), MTBE/CH_2Cl_2 (65/35 : v/v), -78°C, 1h.
Scheme 4

Wittig reaction (Scheme 5) on the aldehyde mixture (**20a** and **20b**) afforded the desired 27-carbon skeleton-steroids **22a** and **22b** (83% yield). The reaction was carried out in THF at -30°C and the ylide was prepared from triphenyl isopropyl phosphonium bromide and sodium amide.

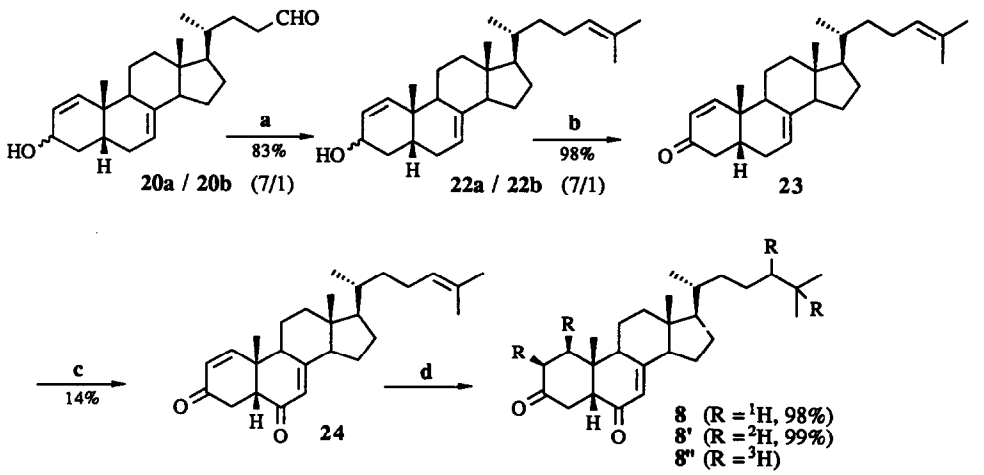
The classical technique for oxidation of the allylic alcohols **22a** and **22b**, with manganese dioxide in chloroform[15], gave the ketone **23** in excellent yield (98%).

Finally, the delicate oxidation in C-6 position was performed using the Collins complex and afforded the diketone **24** in poor yield (14%).

Selective hydrogenation of the Δ^1 and Δ^{24} double bonds using palladium on charcoal afforded the expected compound **8** in 98% yield. The hindered Δ^7 double bond was not reduced under our experimental conditions. Together with the mass spectrometric analysis, ^1H NMR spectrometry clearly demonstrated the reduction of the Δ^1 and Δ^{24} double bond [disappearance of the signals at δ = 1.61 ppm (H-26), 1.68 ppm (H-

27), 5.09 ppm (H-24), 5.87 ppm (H-2) and 6.78 ppm (H-1); appearance of the signal at δ = 0.87 ppm (H-26,27)].

Deuteration was conducted using the same conditions (using D_2 gas) and gave the deuterated diketone in high yield (99%). Again, mass, ^1H NMR and ^2H NMR spectrometry clearly demonstrated the reduction of the Δ^1 and Δ^{24} double bond : Mass spectrometry analysis shows a large isotopic distribution and NMR study indicates that some deuterium atoms are on methyls 26 and 27, due to isotopic exchange under reduction conditions[16]. To determine the precise configuration of the deuteriums introduced in positions C-1 and C-2 (A ring), the deuteriated compound has been reduced using sodium borohydride in methanol to give exclusively the 3-α alcohol[16] (**25**, see experimental part). Under these conditions, the coupling constants of the proton at position C-3β (δ = 3.61 ppm, td, $J_{H3ax-H2eq}$ and $J_{H3ax-H4ax}$ = 10.9 Hz, $J_{H3ax-H4eq}$ = 4.6 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 (a closely related analysis has been described in a preceding paper[17]).



Reagents and conditions: (a) $Ph_3P^+CH(Me)_2\ I^-$, $NaNH_2$, THF, -78°C, then 25°C, 5h; (b) MnO_2 (120 eq), $CHCl_3$, 5h, 25°C; (c) $CrO_3.Py_2$ (20 eq), CH_2Cl_2, 10h, 25°C; (d) H_2 (D_2 or T_2), Pd/C 5%, AcOEt, 25°C.

Scheme 5

Tritiation was performed at the Commissariat à l'Energie Atomique (Saclay, France). Under the two conditions of tritiation used (Pd/C and Wilkinson catalysts) the same compound was obtained but with a better yield when the soluble Wilkinson catalyst is used. The labelled molecule was purified on TLC and by HPLC on silica and on reversed phase columns. As in the deuterated compound, mass spectrometry analysis shows a large isotopic distribution and ^3H NMR study indicates that more than 40% of the radioactivity is associated with methyls 26 and 27. The specific activity of the labelled molecule determined by UV and liquid scintillation counting was 9.2 TBq/mmol (250 Ci/mmol). The labelled compound has the same chromatographic behaviour as the non-radioactive molecule, in TLC and in HPLC on reversed phase and on silica gel columns. Again, some isotopic exchange of methyls under tritiation conditions may be the reason for the observed high specific activity[16].

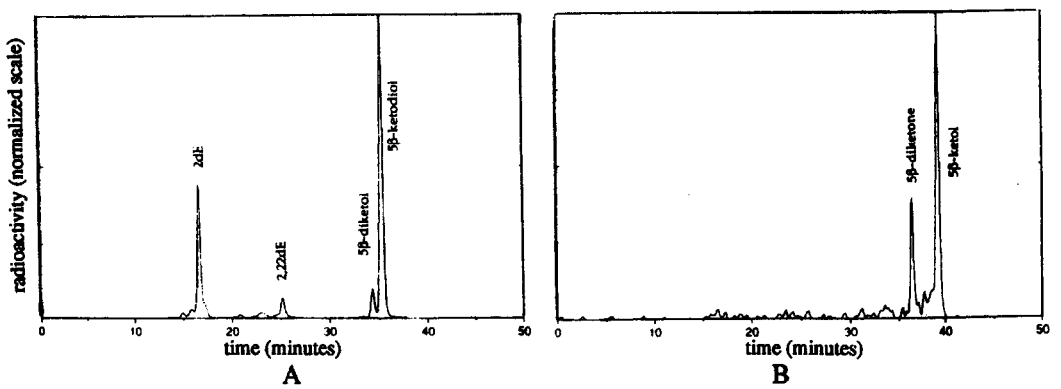
Biological Results

The metabolism of this compound was tested in two different biological systems and compared with that of 5β-diketol : (1) *in vivo* by injection to the Myriapod *Lithobius forticatus*[18] and (2) *in vitro* upon incubation with prothoracic glands of the cabbage butterfly *Pieris barssicae*[19]. In *Lithobius*, it has been shown previously that 5β-diketol (**7**) is efficiently reduced into 5β-ketodiol (**5**), then converted into 2,22-dideoxyecdysone and 2-deoxyecdysone whereas in *Pieris* 5β-diketol (**7**) is not reduced and it is converted into 3-dehydro-22-deoxyecdysone and 3-dehydroecdysone (**2**).

In *Lithobius*, 5β-diketone (**8**) was reduced into 5β-ketol (**6**), but no further conversion was observed, whereas control animals injected with 5β-diketol (**5**) show the whole conversion up to 2-deoxyecdysone (figure 1). Thus, neither 5β-diketone (**8**) nor 5β-ketol (**6**) seem to be substrates of the 22- and 25- hydroxylases, and the presence of a 14α-OH seems to be an absolute requirement.

In *Pieris*, 5β-diketone remained almost unchanged after overnight incubation, whereas 5β-diketol (**5**) was converted in the control experiement. Therefore, in this system the absence of a 14α-OH seems to preclude any further conversion (hydroxylations at 2-, 22- and 25- positions).

These data seem to indicate that 5β-diketone (**8**) is an unlikely intermediate in the ecdysone biosynthetic pathway, and this once again[7] stresses the fact that the 14α-OH must be introduced at a very early stage.



Conversion studies of tritiated compounds *in vivo* by *Lithobius forficatus*. A : Conversion of 14α-hydroxy-5β-cholest-3,6-dione (5β-diketol, **7**). B : Conversion of 5β-cholest-7-ene-3,6-dione (5β-diketone, **8**). 2dE, 2-deoxyecdysone; 2,22dE, 2,22-dideoxyecdysone (for details see text).

Figure 1

Acknowledgments

The authors wish to thank Mr Jean Daniel Sauer and Mrs Elisabeth Krempp for NMR spectra, Dr Gérard Teller for mass spectra. We express our gratitude to Professor Guy Ourisson for critical reading of this manuscript.

	9[§]	10[#]	11[#]	12[#]	13[#]	14[#]	15[#]	16[#]	17[#]	18[#]	19[#]	20a[#]	20b[#]	22a[#]	22b[#]	23[#]	24[§]	8[§]
1	36.6[+]	35.3[+]	36.9[+]	36.2[+]	34.5[+]	48.6	162.3	33.9[+]	153.0	155.1	157.7	141.1	143.3	140.9	143.2	162.1	158.5	35.1[+]
2	31.4	30.6	36.8[+]	37.7[+]	35.5[+]	43.3[+]	127.8	34.0[+]	127.5'	127.9'	128.6	128.5	126.2	128.6	126.2	127.6	129.0	37.4[+]
3	72.9	72.0	213.2	212.6	211.1	202.4	200.7	199.6	186.1	186.4	198.0[+]	69.0	64.7	68.9	64.6	200.4	198.2	208.7
4	35.9[+]	39.6	45.8	43.5	44.2	42.8[+]	40.4	123.6'	123.7'	123.4'	35.4	35.8	34.5	35.8	34.4	40.3	36.1	39.4[+]
5	43.2	41.5	43.2	42.0	43.1	54.9	40.4	163.9	162.7	165.9	51.4	44.5	43.2	44.4	43.1	40.3	52.4	56.9
6	40.5	34.6[+]	33.9	28.3	129.3[°]	27.6	27.7	127.9'	128.1'	32.6	196.4[+]	28.8	28.3	29.7	28.2	27.7	196.7	198.9
7	69.1	68.5	68.5	114.7	128.9[°]	114.7	115.3	141.4	138.6	115.9	121.3	115.7	115.8	115.2	115.4	115.0	121.5	120.7
8	40.8	39.4	39.4	137.9	36.7	137.3	139.0	37.8	38.1	138.1	164.5	137.4	137.6	137.6	137.5	139.1	165.3	165.3
9	34.1	32.8	33.3	35.3	35.2	37.0	43.6	53.4[*]	48.3	47.3	56.2	39.1	34.3	39.1	34.2	43.4	56.8	41.9
10	36.2	35.0	35.3	33.7	33.4	37.3	37.5	36.1	41.2	42.4	39.1	36.0	36.0	36.0	36.0	37.3	39.7	36.9
11	21.8	20.6	21.0	22.2	20.9	22.8	22.8	20.7	21.8	22.8	21.8	22.4	22.6	22.4	22.5	22.5	22.3	22.5
12	41.1	39.8	39.5	39.8	40.0	39.4	39.5	39.5	39.0	39.4	39.0	39.8	39.8	39.8	39.7	39.5	39.7	39.7
13	43.7	42.7	42.7	43.9	43.3	43.6	43.6	43.4	43.0	43.5	46.2	43.7	43.7	43.6	43.5	43.4	46.5	46.7
14	51.5	50.4	50.3	55.2	54.4	53.9	54.6	50.6[*]	54.6	53.5	56.0	54.7	54.7	54.7	54.6	54.5	56.8	55.1
15	24.6	23.7	23.7	22.6	23.8	22.7	22.8	23.7	23.6	23.0	24.0	22.7	22.7	22.9	22.8	22.7	24.9	22.5
16	29.2	28.1	28.1	27.9	28.2	27.8	27.7	28.0	28.0	27.7	27.6	27.9	27.9	27.9	27.8	27.6	28.2	28.2
17	57.4	55.8	55.8	55.8	55.8	55.6	55.7	55.7	55.6	55.6	56.0	55.8	55.8	56.1	56.0	55.9	56.8	56.9
18	12.2	11.7	11.8	11.9	12.0	11.9	11.9	11.9	12.0	11.9	12.3	11.9	11.9	11.9	11.8	11.8	12.5	12.5
19	23.4	22.8	21.9	23.4	21.1	23.3	22.0	16.3	20.7	19.5	22.6	22.8	22.8	22.7	22.5	21.9	23.1	22.9
20	36.7	35.3	35.3	35.7	35.7	35.7	35.7	35.3	35.3	35.6	35.4	36.0	36.0	36.0	35.9	35.9	36.2	36.4
21	18.6	18.3	18.3	18.4	18.3	18.4	18.4	18.2	18.2	18.4	18.3	18.6	18.6	18.8	18.7	18.7	18.8	19.0
22	32.4[-]	31.0[-]	31.0[-]	30.9[-]	30.9[-]	30.7[-]	30.9[-]	30.9[-]	30.8[-]	30.8[-]	30.6[-]	27.9	27.9	36.0	35.9	35.9	36.2	36.4
23	32.0[-]	31.0[-]	31.0[-]	31.1[-]	31.1[-]	31.0[-]	31.1[-]	31.0[-]	31.0[-]	31.0[-]	30.9[-]	41.0	41.0	24.8	24.7	24.7	25.1	24.2
24	178.1	174.7	174.7	174.6	174.6	174.8	174.6	174.6	174.6	174.6	174.3	203.1	203.1	125.2	125.1	125.0	125.3	39.8
25														130.9	130.9	130.8	131.5	28.4
26														17.6	17.6	17.5	17.7	22.7
27														25.7	25.6	25.6	25.8	23.0
1'		51.4	51.5	51.5	51.5	51.6	51.5	51.5	51.5	51.5	51.4							

Table 1 : ^{13}C NMR chemical shifts

δ_C (100 MHz; standard Me_4Si). * (or +, -, ', ",°) : interchangeable assignment. Solvent : (#) $CDCl_3$ (77.02), (§) CD_2Cl_2 (53.80) or ($) CD_3OD (49.01).
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. 17 ; (d) J.W. Blunt *et al.*, *Org. Magn. Reson.*, 1977, *9*, 439.

Experimental

Melting points were measured on a Reichert hot stage microscope and are uncorrected. Optical rotations ($[\alpha]_D$) 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 Bruker SY (200 MHz) and Bruker AM (400 MHz) spectrometer using $CHCl_3$ (δ = 7.26 ppm) and CH_2Cl_2 (δ = 5.32 ppm) as internal standards for ^1H NMR and $CDCl_3$ (δ = 77.02 ppm) or CD_2Cl_2 (δ = 53.80 ppm) as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS (*, $^+$ = interchangeable assignement). Mass spectra (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 (unless otherwise stated) was used. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. TLC were run on pre-coated silica gel plates 60 F 254 (Merck, 0.25 mm), 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 carried out using silica gel (40 - 63 µm, Merck) columns. All solvents were freshly distilled before use. Air- or moisture-sensitive reactions were conducted in flame-dried glassware and under an inert atmosphere.

24-Methyl-3α,7α-dihydroxy-5β-cholanoate (10)

A mixture of chenodeoxycholic acid (9) (25.0 g, 63.7 mmol), methanol (50 ml) and concentrated hydrochloric acid (37%, 7 ml) was refluxed for 10 minutes. The reaction mixture was cooled to room temperature and then poured into ethyl acetate (1 litre). The solution was washed twice with aq. sodium hydroxyde solution (10%), water, brine. The organic phase was dried with sodium sulfate, evaporated under reduced pressure and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate : 50/50 gave 24-methyl-3α,7α-dihydroxy-5β-cholanoate (10), 25.1 g (97%).

10 **Mp** : 66-68°C ; $[\alpha]_D^{20}$: + 11 (MeOH, 1.0) ; **IR** ν (cm^{-1}) : 3680 ; 3634 ; 3441 ; 2954 ; 1732 ; 1440 ; 1373 ; 1163 ; 1081 ; ^1H **NMR** 200 MHz ($CDCl_3$) δ : 0.65 (s, 3H, H-18) ; 0.90 (s, 3H, H-19) ; 0.92 (d, J = 5.98 Hz, 3H, H-21) ; 3.46 (m, $w_{1/2}$ = 20.0 Hz, 1H, H-3) ; 3.66 (s, 3H, H-1') ; 3.85 (bd, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; **MS** m/z (%) : 406 (2.9) (M^+, C_{25} H_{42} O_4) ; 398 (27.8) ; 371 (30.2) ; 370 (100.0) ; 355 (51.2) ; 273 (41.2) ; 255 (61.0) ; 246 (31.1) ; 228 (46.1) ; 213 (57.7) ; 147 (32.1) ; **Microanalysis** : calc for C_{25} H_{42} O_4 (406.6028) C : 73.85 ; H : 10.41 ; found C : 72.53 ; H : 9.87 ; ^{13}C **NMR** in table 1.

24-Methyl-3-oxo-7α-hydroxy-5β-cholanoate (11)

A suspension of platinum oxide (10.0 g) in ethyl acetate (250 ml) was stirred magnetically in an hydrogen atmosphere at room temperature and atmospheric pressure, until reduction to platinum was complete. The hydrogen in the system was then replaced with nitrogen by careful and repeated evacuation*. A solution of 24-methyl-3α,7α-dihydroxy-5β-cholanoate (10) (61.5 mmol, 25.0 g) in ethyl acetate (20 ml) was then added through an attached dropping funnel, and the mixture was stirred for 100 hours with oxygen at room temperature and atmospheric pressure. The reaction mixture was then filtered on celite and the filtrate vaporated under reduce pressure to give pure 24-methyl-3-oxo-7α-hydroxy-5β-cholanoate (11), 24.4 g (98%).

*** In the course of this work, we experienced an explosion and fire, wich was traceable to incomplete removal of hydrogen at this stage. Care should be exercised to wash all catalyst from the walls of the vessel by gentle swirling prior to admitting oxygen.**

11 **Mp** : 128-129°C ; **IR** ν (cm^{-1}) : 3467 ; 2940 ; 1717 ; 1466 ; 1438 ; 1208 ; 1172 ; $[\alpha]_D^{25}$: + 20 ($CHCl_3$, 1.0) ; ^1H **NMR** 200 MHz ($CDCl_3$) δ : 0.70 (s, 3H, H-18) ; 0.94 (d, J = 6.25 Hz, 3H, H-21) ; 1.00 (s, 3H, H-19) ; 3.39 (dd, J = 13.39 Hz and 15.21 Hz, 1H, H-4α) ; 3.66 (s, 3H, H-1') ; 3.92 (bd, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; **MS** m/z (%) : 404 (10.5) (M^+, C_{25} H_{40} O_4) ; 387 (12.9) ; 386 (41.7) ; 371 (15.8) ; 353 (16.3) ; 271 (100.0) ; 229 (21.2) ; 211 (18.0) ; Microanalysis : calc for C_{25} H_{40} O_4 (404.5870) C : 74.22 ; H : 9.96 ; found C : 74.02 ; H : 9.71 ; ^{13}C **NMR** in table 1.

24-Methyl-3-oxo-5β-chol-7-enoate (12)

Phosphorus oxychloride (25 ml) was added at 0°C to a solution of methyl-3-oxo-7α-hydroxy-5β-cholanoate (11) (18.3 g, 45.2 mmol) in dry pyridine (250 ml). The reaction mixture was stirred at room temperature for 16 hours, poured onto ice and extracted twice with ether. The organic layer was washed several times with 0.5N hydrochloric acid, water, aq. sat. sodium hydrogencarbonate, brine and dried with sodium sulfate. The solution was concentrated to dryness under reduce pressure and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate : 90/10 gave 24-methyl-3-oxo-5β-chol-7-enoate (12), 13.3 g (76%) and 24-methyl-3-oxo-5β-chol-6-enoate (13), 2.8 g (16%) as an oil.

12 **Mp** : 117-119°C ; $[\alpha]_D^{25}$: + 23 ($CHCl_3$, 1.1) ; IR ν (cm^{-1}) : 2951 ; 2872 ; 1712 ; 1433 ; 1216 ; 1178 ; **^{1}H NMR** 200 MHz ($CDCl_3$) δ : 0.57 (s, 3H, H-18) ; 0.94 (d, J = 6.07 Hz, 3H, H-21) ; 0.98 (s, 3H, H-19) ; 3.66 (s, 3H, H-1') ; 5.12 (b, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; **MS** m/z (%) : 386 (94.1) (M^+, C_{25} H_{38} O_3) ; 371 (18.8) ; 354 (31.2) ; 353 (100.0) ; 316 (33.2) ; 315 (56.2) ; 271 (19.9) ; 229 (21.6) ; 211 (27.3) ; **Microanalysis** : calc for C_{25} H_{38} O_3 (386.5722) C : 77.68 ; H : 9.91 ; found C : 77.50 ; H : 9.71 ; **^{13}C NMR** in table 1.

13 **^{1}H NMR** 200 MHz ($CDCl_3$) δ : 0.73 (s, 3H, H-18) ; 0.94 (d, J = 6.07 Hz, 3H, H-21) ; 0.94 (s, 3H, H-19) ; 3.66 (s, 3H, H-1') ; 5.46 (B part of an AB system, that looks like a d, J = 10.09 Hz, 1H, H-6) ; 5.59 (A part of an AB system, that looks like a dm, J = 10.10 Hz and $w_{1/2}$ = 10.0 Hz, 1H, H-7) ; **MS** m/z (%) : 386 (100.0) (M^+, C_{25} H_{38} O_3) ; 371 (22.7) ; 354 (24.6) ; 353 (66.0) ; 316 (36.9) ; 315 (47.2) ; 271 (51.8) ; 249 (57.5) ; 229 (43.1) ; 211 (27.1) ; **^{13}C NMR** in table 1.

24-Methyl-3-oxo-5β-chol-1,7-dienoate (15)

First step

To a solution of 24-methyl-3-oxo-5β-chol-7-enoate (12) (7.0 g, 18.1 mmol) in dry tetrahydrofuran (90 ml) was added dropwise at 0°C tri-N-methylanilinium perbromide (7.15 g, 19.0 mmol, 1.05 eq) in tetrahydrofuran (20 ml). After addition (5 minutes), the mixture was stirred for 15 minutes at 0°C and for a further 30 minutes at room temperature The mixture was then poured into water and the product extracted with ether. The organic layer was washed with water, brine, dried with sodium sulfate and evaporated to dryness to yield 24-methyl-2β-bromo-3-oxo-5β-chol-7-enoate (14), 8.25 g (98%), which was used without further purification for the next step. Recrystallization from acetone of an aliquot gave pure 24-methyl-2β-bromo-3-oxo-5β-chol-7-enoate (14) as white needles.

14 Mp : 140-142°C ; $[\alpha]_D^{25}$: 0 ($CHCl_3$, 1.6) ; $[\alpha]_{500}^{25}$: - 7 ($CHCl_3$, 1.6) ; IR ν (cm^{-1}) : 2961 ; 1724 ; 1435 ; 1168 ; **^{1}H NMR** 200 MHz ($CDCl_3$) δ : 0.57 (s, 3H, H-18) ; 0.95 (d, J = 6.12 Hz, 3H, H-21) ; 0.98 (s, 3H, H-19) ; 2.68 (dd, J = 13.18 and 5.96 Hz, 1H, H-1β) ; 3.66 (s, 3H, H-1') ; 4.86 (dd, J = 13.44 and 6.51 Hz, 1H, H-2α) ; 5.13 (bm, $w_{1/2}$ = 10.0 Hz, 1H, H-7) ; **MS** (15eV) m/z (%) : 466 (4.8) (M^+, C_{25} H_{37} O_3 Br) ; 464 (4.6) ; 386 (60.8) ; 385 (100.0) ; 367 (8.6) ; 353 (16.1) ; 327 (16.8) ; 315 (24.2) ; **Microanalysis** : calc for C_{25} H_{37} O_3 Br (365.4643) C : 64.51 ; H : 8.01 ; found C : 62.79 ; H : 7.80 ; **^{13}C NMR** in table 1.

Second step

A solution of the crude bromide (14) (8.25 g, 17.7 mmol) in dimethyl formamide (100 ml) containing lithium carbonate (4.1 g, 55 mmol, 3.1 eq) and lithium bromide (2.7 g) was refluxed for 30 minutes. The solution was cooled to room temperature and diluted with ether. The resulting mixture was washed carefully with 12N hydrochloric acid, water , aq. sat sodium hydrogencarbonate, brine. the organic phase was dried with sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel. Elution with hexane/ethyl acetate : 85/15 gave 24-methyl-3-oxo-5β-chol-1,7-dienoate (15) , 3.95 g (58%), 24-methyl-3-oxo-5β-chol-1,6-dienoate (16), 1.70 g (25%) and a mixture of 24-methyl-3-oxo-5β-chol-1,4,7-trienoate (17) and 24-methyl-3-oxo-5β-chol-1,4,6-trienoate (18), 730 mg (10%).

15 **Mp** : 138-139°C ; $[\alpha]_D^{25}$: + 40 ($CHCl_3$, 0.8) ; **UV** : λ_{max}(CH_3CN) = 196 nm (18200), 229 nm (15300) ; **IR** : ν (cm^{-1}) : 2955 ; 2842 ; 1733 ; 1661 ; 1378 ; 1217 ; 1188 ; **^{1}H NMR** 200 MHz ($CDCl_3$) δ : 0.57 (s, 3H, H-18) ; 0.93 (d, J = 6.15 Hz, 3H, H-21) ; 1.15 (s, 3H, H-19) ; 3.66 (s, 3H, H-1') ; 5.15 (b, $w_{1/2}$ = 10.0 Hz, 1H, H-7) ; 5.89 (d, J = 10.01 Hz, 1H, H-

2) ; 6.99 (d, J = 10.04 Hz, 1H, H-1) ; MS m/z (%) : 384 (30.0) (M^+, C_{25} H_{36} O_3) ; 369 (6.8) ; 353 (9.9) ; 277 (13.9) ; 276 (66.2) ; 269 (35.7) ; 249 (17.4) ; 221 (29.0) ; 208 (27.6) ; 161 (100.0) ; Microanalysis : calc for C_{25} H_{36} O_3 (384.5564) C : 78.08 ; H : 9.43 ; found C : 78.10 ; H : 9.45 ; ^{13}C NMR in table 1.

16 ^1H NMR 200 MHz ($CDCl_3$) δ : 0.74 (s, 3H, H-18) ; 0.92 (d, J = 6.29 Hz, 3H, H-21) ; 1.09 (s, 3H, H-19) ; 3.65 (s, 3H, H-1') ; 5.65 (s, 1H, H-4) ; 6.10 (A and B part of an AB system, that looks like a bs, $w_{1/2}$ = 5.0 Hz, 2H, H-6,7) ; MS m/z (%) : 384 (100.0) (M^+, C_{25} H_{36} O_3) ; 369 (9.4) ; 269 (45.0) ; 249 (24.1) ; 175 (41.4) ; 174 (32.0) ; 173 (17.3) ; 161 (34.8) ; 136 (51.5) ; **Microanalysis** : calc for C_{25} H_{36} O_3 (384.5564) C : 78.08 ; H : 9.43 ; found C : 77.62 ; H : 9.33 ; ^{13}C NMR in table 1.

17 ^1H NMR 200 MHz ($CDCl_3$) δ : 0.61 (s, 3H, H-18) ; 0.92 (d, J = 6.30 Hz, 3H, H-21) ; 1.22 (s, 3H, H-19) ; 2.79 (A part of an AB system, that looks like a bd, J = 19.20 Hz and $w_{1/2}$ = 10.0 Hz, 1H, H-6) ; 3.15 (B part of an AB system, that looks like a bd, J = 19.10 Hz and $w_{1/2}$ = 10.0 Hz, 1H, H-6) ; 3.66 (s, 3H, H-1') ; 5.23 (bm, $w_{1/2}$ = 10.0 Hz, 1H, H-7) ; 6.10 (bs, $w_{1/2}$ = 5.0 Hz, 1H, H-4) ; 6.23 (d, J = 10.00 Hz, 1H, H-2) ; 7.07 (d, J = 10.01 Hz, 1H, H-1) ; MS m/z (%) : 382 (46.6) (M^+, C_{25} H_{34} O_3) ; 267 (19.7) ; 249 (24.5) ; 248 (23.5) ; 233 (100.0) ; 173 (33.8) ; 171 (36.9) ; **Microanalysis** : calc for C_{25} H_{34} O_3 (382.5406) C : 78.49 ; H : 8.96 ; found C : 78.22 ; H : 9.06 ; ^{13}C NMR in table 1.

18 ^1H NMR 200 MHz ($CDCl_3$) δ : 0.77 (s, 3H, H-18) ; 0.93 (d, J = 6.14 Hz, 3H, H-21) ; 1.18 (s, 3H, H-19) ; 3.66 (s, 3H, H-1') ; 5.99 (bs, $w_{1/2}$ = 5.0 Hz, 1H, H-4) ; 6.05 (b, 1H, H-6) ; 6.20 (b, 1H, H-7) ; 6.24 (d, J = 10.30 Hz, 1H, H-2) ; 7.05 (d, J = 10.10 Hz, 1H, H-1) ; MS m/z (%) : 382 (100.0) (M^+, C_{25} H_{34} O_3) ; 367 (21.1) ; 351 (13.0) ; 267 (38.2) ; 265 (16.3) ; 225 (33.1) ; 211 (22.9) ; 209 (19.3) ; 197 (28.6) ; 171 (75.3) ; **Microanalysis** : calc for C_{25} H_{34} O_3 (382.5406) C : 78.49 ; H : 8.96 ; found C : 78.22 ; H : 9.06 ; ^{13}C NMR in table 1.

24-Methyl-3,6-dioxo-5β-chol-1,7-dienoate (19)

24-Methyl-3-oxo-5β-chol-1,7-dienoate (15)(1.0 g, 2.6 mmol) was dissolved in fresly distilled methylene chloride (P_2O_5) (15 ml) at room temperature and under a nitrogen atmosphere. To the stirred solution was added 12.0 g (42 mmol, 16 eq) of CrO_3.(pyridine)$_2$ complex (Collins reagent, freshly prepared) as a slurry in methylene chloride (30 ml). The mixture immediately began turning brown and depositing a tarry precipitate on the sides and bottom of the flask (It was necessary to have the stirrer paddles near the surface of the solution to avoid clogging the paddles with the precipitate). After 17 hours of stirring at room temperature, an additional (12.0 g, 42 mmol, 16 eq) 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, 0.5N hydrochloric acid, dried with sodium sulfate. The solvent was evaporated to dryness and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 70/30 gave 24-methyl-3,6-dioxo-5β-chol-1,7-dienoate (19), 190.0 mg (18%).

19 Mp : 137-139°C ; $[\alpha]_D^{25}$: + 29 ($CHCl_3$, 1.0) ; UV : λ_{max}(CH_3CN) : 194 nm (16700), 224 nm (18600) ; IR ν (cm^{-1}) : 2954 ; 2877 ; 1733 ; 1660 ; 1614 ; 1563 ; 1462 ; 1163 ; ^1H NMR 200 MHz ($CDCl_3$) δ : 0.61 (s, 3H, H-18) ; 0.94 (d, J = 5.70 Hz, 3H, H-21) ; 1.24 (s, 3H, H-19) ; 3.65 (s, 3H, H-1') ; 5.75 (bt, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; 5.93 (d, J = 10.13 Hz, 1H, H-2) ; 6.77 (d, J = 10.15 Hz, 1H, H-1) ; MS m/z (%) : 398 (47.8) (M^+, C_{25} H_{34} O_4) ; 383 (17.5) ; 367 (20.5) ; 290 (16.1) ; 283 (42.2) ; 262 (33.4) ; 257 (17.7) ; 247 (20.7) ; **Microanalysis** : calc for C_{25} H_{34} O_4 (398.5396) C : 75.34 ; H : 8.60 ; found C : 74.12 ; H : 8.10 ; ^{13}C NMR in table 1.

3-Hydroxy-5β-chola-1,7-dien-24-al (**20a**, OH-3α) (**20b**, OH-3β)

The crude ester **15** (8.5 g, 22.1 mmol) was dissolved in dry distilled *tert*-butyl methyl ether and methylene chloride (460 ml, 35/65 : v/v) at - 78°C and the mixture stirred magnetically. Diisobutylaluminium hydride (45 ml, 1M solution in hexane, 2 eq, Aldrich) was added at - 78°C over a period of 30 minutes and the mixture stirred for 1 hour and then quenched at the same

temperature (- 78°C) with 30 ml of an aq. 50% acetic acid solution. The cooling bath was removed and the mixture stirred for 1 hour. The solution was washed with aq. 10% sodium hydroxide solution, water, brine. The organic phase was dried with sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 75/25 gave 3-hydroxy-5β-chola-1,7-dien-24-al (**20a/20b** : 7/1), 7.25 g (92%) and 5β-chola-1,7-dien-3,24-diol (**21a/21b** : 7/1), 200.0 mg (2.5%).

20 **IR** : ν (cm^{-1}) : 3372 ; 2953 ; 2884 ; 1723 ; 1442 ; 1378 ; 1019 ; **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 0.55 (s, 6H, H-18) ; 0.93 (d, J = 6.47 Hz, 6H, H-21) ; 0.97 (s, 3H, H-19 (20a)) ; 1.01 (s, 3H, H-19 (20b)) ; 4.16 (bm, $w_{1/2}$ = 10.0 Hz, 1H, H-3α (20b)) ; 4.36 (bm, $w_{1/2}$ = 20.0 Hz, 1H, H-3β (20a)) ; 5.14 (bs, $w_{1/2}$ = 10.0 Hz, 2H, H-7) ; 5.52 (d, J = 9.96 Hz, 1H, H-1 (20a)) ; 5.64 (dd, J = 10.32 et 4.28 Hz, 1H, H-2 (20b)) ; 5.82 (dd, J = 10.01 and 1.74 Hz, 1H, H-2 (20a)) ; 5.95 (d, J = 9.82 Hz, 1H, H-1 (20b)) ; 9.77 (t, J = 1.69 Hz, 2H, H-24) ; **MS** (20a) m/z (%) : 356 (32.7) (M^+, C_{24} H_{36} O_2) ; 341 (5.3) ; 269 (36.4) ; 248 (84.6) ; 193 (17.8) ; 189 (15.6) ; 161 (90.2) ; (20b) m/z (%) : 356 (5.6) (M^+, C_{24} H_{36} O_2) ; 339 (15.2) ; 338 (52.4) ; 324 (8.1) ; 323 (31.4) ; 310 (7.3) ; 284 (9.3) ; 253 (22.4) ; 246 (51.2) ; 226 (5.3) ; 218 (18.2) ; 199 (6.9) ; 162 (17.7) ; 161 (100.0) ; **Microanalysis** : calc for C_{24} H_{36} O_2 (356.5464) C : 80.85 ; H : 10.18 ; found C : 80.63 ; H : 10.08 ; **^{13}C NMR** in table 1.

21 **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 0.56 (s, 6H, H-18) ; 0.94 (d, J = 6.47 Hz, 6H, H-21) ; 0.97 (s, 3H, H-19 (21a)) ; 1.01 (s, 3H, H-19 (21b)) ; 2.50 (dm, J = 20.0 Hz and $w_{1/2}$ = 20.0 Hz, 2H, H-5) ; 3.62 (bm, $w_{1/2}$ = 20.0 Hz, 4H, H-24) ; 4.16 (bm, $w_{1/2}$ = 10.0 Hz, 1H, H-3α (21b)) ; 4.36 (bm, $w_{1/2}$ = 20.0 Hz, 1H, H-3β (21a)) ; 5.14 (bs, $w_{1/2}$ = 10.0 Hz, 2H, H-7) ; 5.52 (d, J = 9.89 Hz, 1H, H-2 (21a)) ; 5.68 (dd, J = 9.78 et 4.42 Hz, 1H, H-2 (21b)) ; 5.83 (d, J = 9.91 Hz, 1H, H-1 (21a)) ; 5.96 (d, J = 9.88 Hz, 1H, H-1 (21b)) ; **MS** (21a) m/z (%) : 358 (17.7) (M^+, C_{24} H_{38} O_2) ; 340 (37.4) ; 325 (27.9) ; 253 (21.4) ; 248 (49.3) ; (21b) m/z (%) : 358 (4.1) (M+, C_{24} H_{38} O_2) ; 341 (30.6) ; 340 (100.0) ; 326 (16.3) ; 325 (55.2) ; 286 (14.6) ; 253 (49.6) ; 248 (29.6) ; 227 (40.7) ; 220 (19.9) ; 211 (14.3) ; 199 (25.2) ; **Microanalysis** : calc for C_{24} H_{38} O_2 (poids moléculaire 358.5622) C : 80.39 ; H : 10.68 ; found C : 79.90 ; H : 10.60.

5β-Cholesta-1,7,24-trien-3-ol (**22a**, OH-3α) (**22b**, OH-3β)

A suspension of isopropyl(triphenyl)phosphonium bromide and sodium amide (Fluka, Instant ylide, 50 g, ready for use mixture for the preparation of the corresponding ylide, 1 g contains ≈ 2.3 mmol phosphonium bromide) in tetrahydrofuran (300 ml) was stirred under argon for 30 minutes at - 78°C (strong red coloration indicated the formation of ylide) and at room temperature for a further 30 minutes. A solution of 3-hydroxy-5β-chola-1,7-dien-24-al (**20a/20b** : 7/1) (21 mmol, 7.2 g) in tetrahydrofuran (50 ml) was then added dropwise to the solution of the ylide at - 78°C and the combined mixture was then allowed to reach room temperature. After 5 hours, the reaction was stopped by addition of acetic acid (6 ml) in order to neutralise the excess of ylide. The white suspension was then treated with $NaHCO_3$ (30 g) for 1 hour with vigorous stirring. The crude mixture was filtered on celite and washed with ethyl acetate. The combined filtrates were evaporated to dryness and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 85/15 gave 5β-cholesta-1,7,24-trien-3-ol (**22a/22b** : 7/1) , 6.4 g (83%).

22 **IR** ν (cm^{-1}) : 3253 ; 2943 ; 2873 ; 1443 ; 1044 ; **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 0.55 (s, 6H, H-18) ; 0.94 (d, J = 6.47 Hz, 3H, H-21) ; 0.97 (s, 3H, H-19 (22a)) ; 1.01 (s, 3H, H-19 (22b)) ; 1.60* (s, 6H, H-26) ; 1.68* (s, 6H, H-27) ; 4.16 (bm, $w_{1/2}$ = 10.0 Hz, 1H, H-3α (22b)) ; 4.36 (bm, $w_{1/2}$ = 20.0 Hz, 1H, H-3β (22a)) ; 5.09 (t, J = 7.50 Hz, 2H, H-24) ; 5.14 (bs, $w_{1/2}$ = 10.0 Hz, 2H, H-7) ; 5.52 (d, J = 9.95 Hz, 1H, H-1 (22a)) ; 5.64 (dd, J = 10.32 and 4.28 Hz, 1H, H-2 (22b)) ; 5.82 (dd, J = 10.00 and 1.74 Hz, 1H, H-2 (22a)) ; 5.94 (d, J = 9.82 Hz, 1H, H-1 (22b)) ; **MS** (22a) m/z (%) : 382 (1.7) (M^+, C_{27} H_{42} 0) ; 365 (33.7) ; 364 (100.0) ; 350 (12.0) ; 349 (39.8) ; 272 (16.4) ; 253 (21.8) ; 244 (15.8) ; 227 (32.9) ; 211 (11.6) ; **Microanalysis** : calc for C_{27} H_{42} 0 (382.6278) C : 84.76 ; H : 11.06 ; found C : 84.87 ; H : 10.97 ; **^{13}C NMR** in table 1.

5β cholesta-1,7,24-trien-3-one (**23**)

5β-Cholesta-1,7,24-trien-3-ol (**22a/22b**)(6.35 g, 16.6 mmol) was dissolved in chloroform (500 ml) and manganese dioxide (170.0 g, 2.0 mol, 120 eq, Merck, activated precipitate) was added. The reaction mixture was stirred for 5 hours at room temperature and then the suspension was filtered on celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel. Elution with hexane/ethyl acetate 90/10 gave 5β cholesta-1,7,24-trien-3-one (**23**), 6.2 g (98%).

23 **Mp** : 70-71 °C ; $[\alpha]_D^{25}$: + 36 ($CHCl_3$, 1.4) ; **UV** : λ_{max} (CH_3CN) : 200 nm (11800) ; 228 nm (17900) ; **IR** ν (cm^{-1}) : 2956 ; 1677 ; 1453 ; 1376 ; ^{1}**H NMR** 400 MHz ($CDCl_3$) δ : 0.57 (s, 3H, H-18) ; 0.94 (d, J = 6.57 Hz, 3H, H-21) ; 1.15 (s, 3H, H-19) ; 1.60* (s, 3H, H-26) ; 1.68* (s, 3H, H-27) ; 2.44 (dm, J = 14.02 and 10.0 Hz, 1H, H-5) ; 5.09 (t, J = 7.13 Hz, 1H, H-24) ; 5.16 (bs, $w_{1/2}$ = 10.0 Hz, 1H, H-7) ; 5.89 (d, J = 9.99, 1H, H-2) ; 6.99 (d, J = 9.88 Hz, 1H, H-1) ; **MS** m/z (%) : 380 (23.7) (M^+, C_{27} H_{40} 0) ; 356 (11.3) ; 296 (14.4) ; 272 (13.7) ; 269 (14.4) ; 268 (23.1) ; 267 (100.0) ; 206 (26.1) ; 159 (46.7) ; **Microanalysis** : calc for C_{27} H_{40} 0 (380.6120) C : 85.20 ; H : 10.59 ; found C : 85.31 ; H : 10.70 ; ^{13}**C NMR** in table 1.

5β-Cholesta-1,7,24-triene-3,6-dione (24)

5β Cholesta-1,7,24-trien-3-one (23)(1.0 g, 2.6 mmol) was dissolved in fresly distilled methylene chloride (P_2O_5)(100 ml) at room temperature and under a nitrogen atmosphere. To the stirred solution was added CrO_3.(pyridine)$_2$ complex (7.5 g, 26.25 mmol, 10.1 eq, Collins reagent, freshly prepared) as a slurry in methylene chloride (20 ml). The mixture immediately began turning brown and depositing a tarry precipitate on the sides and bottom of the flask (It was necessary to have the stirrer paddles near the surface of the solution to avoid clogging the paddles with the precipitate). After 8 hours of stirring at room temperature, an additional 7.5 g (26.25 mmol, 10.1 eq) of CrO_3.(pyridine)$_2$ complex was added to the reaction mixture. 2 hours later, the reaction was interrupted and the mixture was poured from the flask. The precipitate remaining in the flask was rinsed with small portions of ether. The organic layer was washed several times with aq. sat. sodium hydrogen-carbonate, 0.5N 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 70/30 gave 5β cholesta-1,7,24-trien-3-one (23), 600.0 mg (starting material) and a mixture containing the desired product and two further products wich were not identified (126.0 mg). 5β-Cholesta-1,7,24-triene-3,6-dione (24), 58.0 mg (14%, based on recovered starting material), was purified by preparative TLC (Merck, silica gel 60F_{254} pre-coated ; layer thickness 0.5 mm ; elution solvent : hexane/ethyl acetate 60/40).

24 **Mp** : 124-125 °C ; $[\alpha]_D^{25}$: + 56 ($CHCl_3$, 0.6) ; **UV** : λ_{max} (CH_3CN) : 198 nm (15400) ; 224 nm (18600) ; **IR** ν (cm^{-1}) : 2948 ; 2935 ; 2867 ; 1655 ; 1618 ; 1446 ; 1376 ; 1117 ; ^{1}**H NMR** 400 MHz ($CDCl_3$) δ : 0.62 (s, 3H, H-18) ; 0.97 (d, J = 6.57 Hz, 3H, H-21) ; 1.26 (s, 3H, H-19) ; 1.61* (s, 3H, H-26) ; 1.68* (s, 3H, H-27) ; 5.09 (t, J = 7.10 Hz, 1H, H-24) ; 5.72 (t, J = 1.74 Hz, 1H, H-7) ; 5.87 (d, J = 10.13, 1H, H-2) ; 6.78 (d, J = 10.23 Hz, 1H, H-1) ; **MS** m/z (%) : 394 (58.5) (M^+, C_{27} H_{38} 0_2) ; 379 (19.2) ; 310 (27.2) ; 281 (33.3) ; 257 (32.1) ; 243 (23.5) ; **Microanalysis** : calc for C_{27} H_{38} 0_2 (394.5956) C : 82.18 ; H : 9.71 ; found C : 81.95 ; H : 9.52 ; ^{13}**C NMR** in table 1.

5β-Cholest-7-ene-3,6-dione (8)

The compound **24** (29.3 mg, 74 μmol) was dissolved in ethyl acetate (6 ml). Hydrogenation was conducted over palladium (5%) on activated charcoal (15.0 mg) at room temperature and atmospheric pressure during 35 minutes. The reaction mixture was filtered and evaporated to dryness to give 5β-cholest-7-ene-3,6-dione (**8**), 29.3 mg (99%).

8 **Mp** : 185-187°C ; $[\alpha]_D^{25}$: + 60 ($CHCl_3$, 0.3) ; **UV** : λ_{max} (CH_3CN) : 193 nm (14700) ; 245 nm (18400) ; **IR** ν (cm^{-1}) : 2963 ; 2919 ; 2871 ; 1718 ; 1651 ; 1379 ; 1148 ; ^{1}**H NMR** 200 MHz ($CDCl_3$) δ : 0.63 (s, 3H, H-18) ; 0.87 (d, J = 6.55 Hz, 6H, H-26,27) ; 0.95 (d, J = 4.92 Hz, 3H, H-21) ; 1.11 (s, 3H, H-19) ; 5.73 (bs, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; ^{1}**H NMR** 400 MHz (CD_2Cl_2) δ : 0.63 (s, 3H, H-18) ; 0.87 (d, J = 6.50 Hz, 6H, H-26,27) ; 0.95 (d, J = 5.99 Hz, 3H, H-21) ; 1.10 (s, 3H, H-19) ; 5.67 (bt, J = 1.73 Hz, 1H, H-7) ; **MS** m/z (%) : 398 (100.0) (M^+, C_{27} H_{42} 0_2) ; 383 (37.1) ; 285 (58.8) ; 283 (40.5) ; 259 (61.6) ; 207 (32.6) ; 161 (34.2) ; **Microanalysis** : calc for C_{27} H_{42} 0_2 (398.6268) C : 81.35 ; H : 10.62 ; found C : 81.19 ; H : 10.50 ; ^{13}**C NMR** in table 1.

[Deuteriated]-5β-cholest-7-ene-3,6-dione (1β,2β,24,25-$^2H_4$, 26,27-^2H_n) (**8'**)

The previously described procedure was carried out (with 10.0 mg of **24**, 5.0 mg of Palladium (5%) on activated charcoal and 5 ml of ethyl acetate) under a deuterium atmosphere, to give pure [1β,2β,24,25-$^2H_4$, 26,27-^2H_n]-5β-cholest-7-ene-3,6-dione (**8'**), 9.9 mg (99%).

8' ^{1}**H NMR** 400 MHz (CD_2Cl_2) δ : 0.63 (s, 3H, H-18) ; 0.86 (s, H-26,27) ; 0.95 (d, J =5.94 Hz, 3H, H-21) ; 1.10 (s, 3H, H-19) ; 5.67 (bs, $w_{1/2}$ = 5.0 Hz, 1H, H-7) ; ^{2}**H NMR** 61.4 MHz (CD_2Cl_2) δ : 0.83 (bs) ; 1.10 (m) ; 1.48 (m) ; 2.38 (m) ; MS m/z (%) : 414 (1.0) ; 413 (2.5) ; 412 (3.5) ; 411 (7.0) ; 410 (14.5) ; 409 (26.3) ; 408 (43.6) ; 407 (45.2) ; 406 (32.6) ; 405 (22.1) ; 404 (14.7) ; 379 (15.3) ; 378 (22.4) ; 377 (22.3) ; 287 (16.3) ; 286 (42.1) ; 285 (20.0) ; 251 (36.3) ; 250 (60.1) ; 249 (29.9) ; 161 (100.0).

[Tritiated]-5β-Cholest-7-ene-3,6-dione (8")

Tritiation (Commissariat à l'Energie Atomique, Saclay, France) was performed under a tritium atmosphere, using the same procedure as above and also with another catalyst : the Wilkinson catalyst.

Palladium on activated charcoal : Compound **24** (2.9 mg, 7.4 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 minutes. The solution was filtered and evaporated to dryness. The labile tritium was eliminated by dilution in methanol and evaporation, twice.

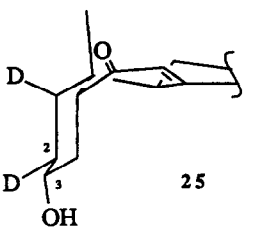
Wilkinson catalyst : $(Ph_3P)_3RhCl$: Compound **24** (2.9 mg, 7.4 µmol) was dissolved in toluene (3 ml). Tritiation (tritium gas : 0.67 TBq, 20 Ci) was conducted (via a Toepler pump) over $(Ph_3P)_3RhCl$ (5.0 mg) at room temperature and at 500 mmHg pressure during 55 minutes. The solution was filtered on silica gel (63 - 200 µm, Merck) and concentrated to dryness. The labile tritium was eliminated by dilution in methanol and evaporation, twice.

Compound **8"** was obtained with a radiochemical purity about 97% by High Performance Liquid Chromatography on Silica (Zorban ODS, Methanol/H_2O : 90/10, 1 ml/mn) and on Reversed Phase columns (Zorban SiO_2, heptane/isopropanol : 99/1, 1 ml/mn). The specific acitvity of the labelled molecule was determined by UV and liquid scintillation counting.

8" ^{3}**H NMR** 320 MHz (Bruker C300) ($CDCl_3$) δ : 0.72 (d) ; 1.03 (m) ; 1.40 (m) ; 1.47 (s) ; 2.29 (d) ; MS (DCI, NH_3) (Finnigan 4600) m/z (%) : 438 (40) ; 436 (46) ; 434 (41) ; 432 (31) ; 430 (41) ; 429 (26) ; 428 (34) ; 426 (39) ; 422 (23) ; 224 (28) ; 188 (46) ; 136 (33) ; 69 (100) ; UV : λ_{max} (CH_3CN) : 243.3 nm (17300) ; Specific activity : 9.2 TBq/mmol or 250 Ci/mmol.

[1β,2β,24,25-$^2H_4$, 26,27-^2H_n]-3α-Hydroxy-5β-cholest-7-en-6-one (25)

To a solution of [1β,2β,24,25-$^2H_4$, 26,27-^2H_n]-5β-Cholest-7-ene-3,6-dione (**8'**) (1.0 mg, 2.5 µmol) in methanol (1 ml) was added 1.0 mg of sodium borohydride. The mixture was stirred 5 minutes, then 1 ml of water was added. Extraction with ether gave [1β,2β,24,25-$^2H_4$, 26,27-^2H_n]-3α-Hydroxy-5β-cholest-7-en-6-one (**25**), 0.5 mg (50 %).



25

25 ^{1}**H NMR** 400 MHz (CD_2Cl_2) δ : 0.61 (s, 3H, H-18) ; 0.91 (s, H-26,27) ; 0.95 (d, J =6.23 Hz, 3H, H-21) ; 1.26 (s, 3H, H-19) ; 5.62 (bs, $w_{1/2}$ = 5.0 Hz, 1H, H-7).

Biological Techniques

Groups of 4-6 *Lithobius forficatus* were anaesthetized with CO_2, then animals were injected each with 1 µl ethanol containing the compound to be tested (ca 0.1 µCi). Animals were then kept at room temperature, then extracted with 100% methanol (10 ml, twice). The extract was centrifuged then evaporated to dryness, and an aliquot was used for HPLC analysis.

Seven prothoracic glands from *Pieris brassicae* larvae, at the end of the last instar, were incubated overnight at 22°C in 200 µl Grace's medium containing 0.5 µCi 5β-diketol or 1 µCi 5β-diketone (contralateral glands from the same animals). The incubation medium was extracted twice by methanol, then an aliquot was submitted to HPLC.

HPLC separation was performed with a Waters HPLC system and a Spherisorb 5ODS2 column (250 mm long 4.6 mm i.d.). Elution used a linear gradient (over 30 min) from 20% to 100% acetonitrile-isopropanol (5/2) in 0.1% trifluoroacetic acid in

water during 30 min, followed by 20 min isocratic conditions with acetonitrile-isopropanol (5/2). Radioactivity was measured in-line with a radioactivity monitor (Radiomatic Flo-one beta A200) using a cell volume of 0.5 ml and 3 ml/min scintillation cocktail (Ecoscint LS271 from National Diagnostics).

References

1. Warren, J.T.; Sakurai, S.; Rountree, D.B.; Gilbert, L.I.; Lee, S.-S. and Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 958-962.
2. Spaziani, E.; Rees, H.H.; Wang, W.L. and Watson, R.D. *Mol. Cell. Endocrinol.* **1989**, *66*, 17-25.
3. Dollé, F.; Kappler, C.; Hetru, C.; Rousseau, B.; Coppo, M.; Luu, B.and Hoffmann, J.A. *Tetrahedron* **1990**, *46*, 5305-5316.
4. Warren, J.T. and Hetru, C. *Invertebrate Reproduction and development* **1990**, *18*, 91-99.
5. Grieneisen, M.L.; Warren, J.T.; Sakurai, S. and Gilbert, L.I. *Insect. Biochem.* **1991**, *21*, 41-51.
6. Hetru, C.; Kappler, C.; Hoffmann, Jules A.; Nearn, R.; Luu, B. and Horn, D.H.S. *Mol. Cell. Endocrinol.* **1982**, *26*, 51-80.
7. Haag, T.; Meister, M.-F.; Hetru, C.; Kappler, C.; Nakatani, Y.; Beaucourt, J.P.; Rousseau, B. and Luu, B. *Insect. Biochem.* **1987**, *17*, 291-301.
8. Dollé, F.; Hetru, C.; Roussel, J.P.; Rousseau, B.; Sobrio, F.; Luu, B.and Hoffmann, J.A. *Tetrahedron* **1991**, *47*, 7067-7080.
9. Lockeley, W.J.S.; Boid, R.; Lloyd-Jones, J.G.; Rees, H.H. and Goodwin, T.W. *J. Chem. Soc., Chem. Commun.* **1975**, 346-348.
10. Davies, T.G.; Dinan, L.N.; Lockeley, W.J.S.; Rees, H.H. and Goodwin, T.W. *Biochem. J.* **1981**, *194*, 53-62.
11. Sneeden, R.P.A. and Turner R. *J. Am. Chem. Soc.* **1955**, *77*, 190-191.
12. Levy, D. and Stevenson, R. *J. Org. Chem.* **1968**, *33*, 2804-2807.
13. Emke, A.; Hands, D.; Midgley, J.M.; Whalley, W.B.; Ahmad, R. *J. Chem. Soc. Perkin Trans I* **1976**, 820-822.
14. Dauben, W.G.; Lorber, M.; Fullerton, D.S. *J. Org. Chem.* **1969**, *34*, 3587-3592.
15. Dollé, F.; Hetru, C. and Luu *Tetrahedron* **1991**, *47*, 7059-7066.
16. Elvidge, J.A.; Jones, J.R.; Lenk, R.M.; Tang, Y.S.; Evans, E.A.; Guilford, G.L. and Warrell, D.C. *J. Chem. Research.* **1982**, 82-83.
17. Dollé, F.; Hetru, C.; Roussel, J.-P.; Rousseau, B.; Sobrio, F.; Luu, B.and Hoffmann, J.A. *Tetrahedron* **1991**, *47*, 7067-7080.
18. Descamps, M. and Lafont, R. *Insect Biochem. Molec. Biol.* **1992**, submitted.
19. Blais, C. and Lafont, R. *C. R. Acad. Sci. Paris* **1991**, *313*, 359-364.