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Biosynthesis of 20-Hydroxyecdysone in *Ajuga* Hairy Roots: Fate of 6α- and 6β-Hydrogens of Lathosterol

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Abstract—The fate of 6α- and 6β-hydrogens of lathosterol during the transformation into 20-hydroxyecdysone was chased by feeding [3α,6β-$^2H_2$]- and [3α,6α-$^2H_2$]-lathosterols to hairy roots of *Ajuga reptans* var. *atropurpurea*. The behavior of 6β-hydrogen, which mostly migrated to the C-5 position of 20-hydroxyecdysone, was in agreement with that of C-6 hydrogen of cholesterol. The results strongly supported the view that cholesterol and lathosterol are first metabolized into 7-dehydrocholesterol, which is then converted into 20-hydroxyecdysone via 7-dehydrocholesterol 5α,6α-epoxide in the hairy roots. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

20-Hydroxyecdysone (**1**) is a molting hormone of most arthropods and it is also distributed in the plant kingdom. The structure of the steroidal hormone is characterized by polyhydroxyl groups, a 7-en-6-one conjugated system and a *cis*-A/B-ring junction. It is well documented that cholesterol (**2**) serves as a biosynthetic precursor of the hormone in both insects and plants.[1] However, the mechanism of the early stages in 20-hydroxyecdysone biosynthesis (i.e. the mechanism of the formation of the 5β-H-7-en-6-one structure) has remained uncertain.[1–3]

In previous papers, we reported that 3α, 4α, and 4β-hydrogens of **2** are retained at the original positions after the conversion into **1**,[4] and that 3β-hydroxy-5β-cholest-7-en-6-one (5β-ketol) (**3**) is converted into **1**[4] in hairy roots of *Ajuga reptans* var. *atropurpurea*.[5,6] Recently, we have found that most of C-6 hydrogen of cholesterol migrates to the C-5 position of **1**.[7] On the basis of these findings, together with the obligatory intermediacy of 7-dehydrocholesterol **4** in insects,[1–3] we proposed a pathway, **2**→**4**→**5**→**3**→**1** (Scheme 1), in *Ajuga* hairy roots. An intermediary role of 7-dehydrocholesterol 5α,6α-epoxide **5** has been previously postulated by our[8] and other[9,10] groups.

Interestingly, the metabolic fates of 3α-, 4α-, 4β- and 6-hydrogens of **2** are different between *Ajuga* hairy roots, the insects *Schistocerica gregaria*[11] and the fern *Polypodium vulgare*.[12] This suggests that three distinct mechanisms are operating in the construction of the 5β-H-7-en-6-one structure of 20-hydroxyecdysone. Further studies on the mechanism of the early stages of ecdysteroid biosynthesis are thus required.

Adler and his co-workers have demonstrated that lathosterol (**6**) is incorporated into **1** in spinach leaves without information of the intermediary role of 7-dehydrocholesterol.[13] We expected that feeding studies of labeled lathosterols would provide a clue to the mechanism of the early stages of 20-hydroxyecdysone biosynthesis. The present paper describes the fate of 6α- and 6β-hydrogens of **6** during the conversion into **1**. In addition, a feeding study of a postulated intermediate, 7-dehydrocholesterol, is described.

Results and Discussion

A preliminary feeding of [3α-^{2}H]lathosterol[14] to *Ajuga* hairy roots showed that **6** is incorporated into **1** as effectively as [3α-^{2}H]cholesterol. Then, [6β-^{2}H]lathosterol (**6a**) (for the synthesis, see Scheme 2) was fed to see the fate of 6β-hydrogen. The ^{2}H NMR spectrum of the biosynthesized **1** exhibited a signal at δ 2.95 (data not shown), which corresponds to the 5-H signal of **1**. The results indicated that 6β-hydrogen of **6** migrates to the C-5 position of **1** during the conversion. The fate of 6β-hydrogen of **6** coincides with that reported for C-6 hydrogen of **2**.[7] The observed 1,2-hydrogen migration ruled out a direct C-6 oxidation pathway of **6** leading to

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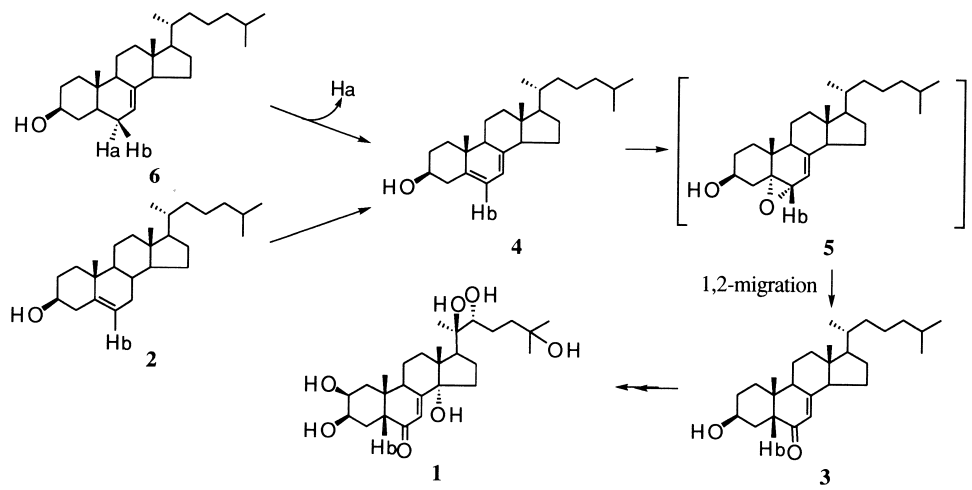
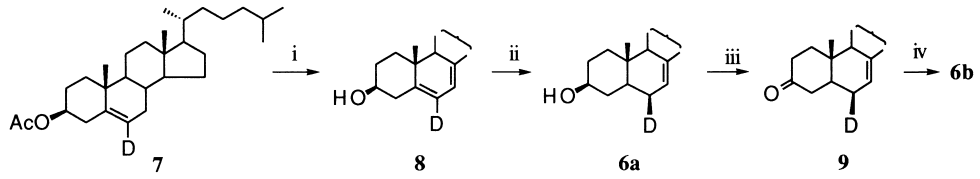


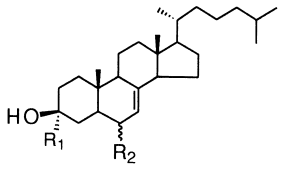
Scheme 1. Conversion of cholesterol (**2**) and lathosterol (**3**) into 20-hydroxyecdysone (**1**) via 7-dehydrocholesterol in *Ajuga* hairy roots with 1,2-shift of hydrogen (Hb) from C-6 to C-5.



Scheme 2. Synthesis of [3α,6β-$^2H_2$]lathosterol. Reagents and conditions: (i) NBS; Bu_4NBr, Bu_4NF; KOH-MeOH (32%), (ii) Raney Ni (91%), (iii) PCC (91%), (iv) $LiAlD_4$ (71%).

3, since the route cannot account for the behavior of 6β-hydrogen of **6**. This implies that **6** has to be converted into **4**, which would serve as a substrate for the subsequent oxidation leading to the epoxide **5**.

We have recently reported that ca 70% of C-5 hydrogen of **1** originates from C-6 hydrogen of **2**, whereas the remaining (30%) comes from another source, presumably from water.[7] If the fate of 6β-hydrogen of **6** is the same as that of C-6 hydrogen of **2**, it would further substantiate the obligatory intermediacy of **4**. To obtain such a quantitative information, [3α,6β-$^2H_2$]- and [3α,6α-$^2H_2$]-lathosterols (**6b** and **6c**) were synthesized and subjected to the feeding. The doubly deuterium-labeled substrates were used to eliminate a possible deuterium isotope effect.



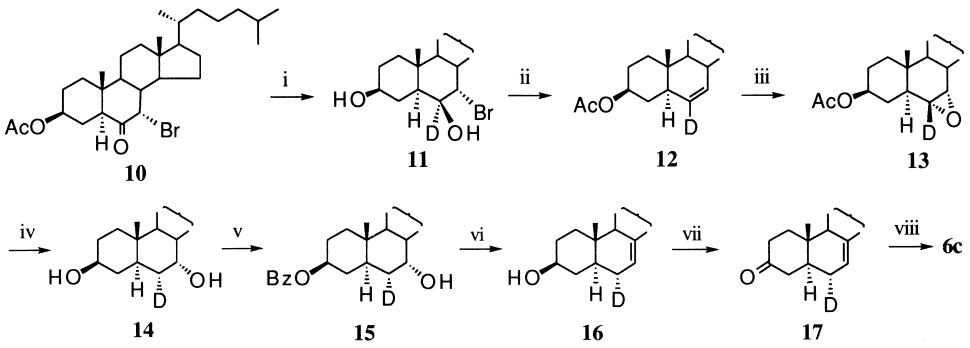
6a: R_1=H, R_2=β-^{2}H
6b: R_1=^{2}H, R_2=β-^{2}H
6c: R_1=^{2}H, R_2=α-^{2}H

The synthesis of **6b** is outlined in Scheme 2. [6-^{2}H]Cholesterol acetate (**7**) was converted into [6-^{2}H]-7-dehydrocholesterol (**8**) according to the method of Rappoldt et al.[15] Hydrogenation of **8** with Raney nickel afforded [6β-^{2}H]lathosterol (**6a**).[16,17] This was converted into [3α,6β-$^2H_2$]lathosterol (**6b**) via 3-ketone (**9**).

[3α,6α-$^2H_2$]Lathosterol (**6c**) was synthesized from the known bromoketone (**10**)[16] according to Scheme 3. Thus, compound **10** was converted into [6α-^{2}H]-3β,7α-diol (**14**) via bromohydorin **11**, 6-ene **12** and 6α,7α-epoxide **13**, according to a slight modification of the published method.[18,19] Mono-benzoylation of **14** gave **15** which, upon dehydration, afforded [6α-^{2}H]lathosterol **16**. Compound **6c** was obtained from **16** via 3-ketone **17** in the same manner as described for the conversion of **6a** to **6b**.

The two labeled sterols, **6b** and **6c**, were separately fed to *Ajuga* hairy roots. The ^{2}HNMR spectra of the resulting 20-hydroxyecdysone (**1**) are shown in Figure 1. 20-Hydroxyecdyone derived from **6b** exhibited two peaks at δ 2.95 and 4.18 in the ratio of ca 7:10, which correspond to 5β-H and 3α-H of **1**, respectively. In contrast, **1** derived from **6c** showed a single peak at δ 4.18. The results unambigously established the loss of 6α-hydrogen of **6** and the migration of 6β-hydrogen of **6** to the C-5 position of **1**. This is in accord with the known steric course, i.e. *cis*-elimination in the formation of Δ^5-sterol from Δ^7-sterol in plants.[20–22] More importantly, the behavior of 6β-hydrogen of **6** (ca 70% shifted to C-5 of **1**) is in excellent agreement with that of C-6 hydrogen of **2**. The whole data described above further supported that **2** and **6** once lead to **4** which, in turn, is transformed into **1** in *Ajuga* hairy roots and presumably in spinach leaves.

The sterol fraction was separated from the hairy roots in the above experiments and cholesterol was isolated



Scheme 3. Synthesis of [3α,6α-$^2H_2$]lathosterol. Reagents and conditions: (i) $LiAlD_4$ (34%), (ii) Zn, AcOH; Ac_2O/Py (81%), (iii) mCPBA (99%), (iv) $LiAlH_4$(100%), (v) BzCl/Py (73%), (vi) $POCl_3$/Py; KOH, MeOH (38%), (vii) PCC (71%), (viii) $LiAlD_4$ (86%).

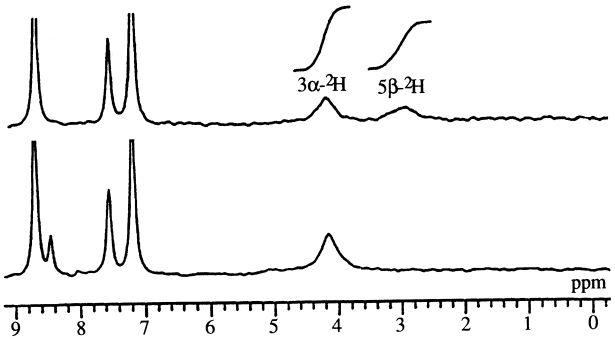


Figure 1. The ^{2}H-NMR spectra (77 MHz, pyridine) of 20-hydroxyecdysone (**1**). Top: derived from [3α,6β-$^2H_2$]lathosterol (**6b**); Bottom: derived from [3α,6α-$^2H_2$]lathosterol (**6c**). The three intense signals at δ 7.19, 7.56 and 8.69 are those of the solvent.

by HPLC. The ^{2}HNMR spectrum of cholesterol derived from **6b** showed two signals at δ 3.51 and 5.35 due to 3-^{2}H and 6-^{2}H, respectively, in equal intensity, whereas that from **6c** showed a single signal at δ 3.51. This firmly established that the elimination of 6α-hydrogen is highly stereospecific and no loss of 6β-hydrogen of **6** takes place during the conversion of **6** into **2** via **4**. Moreover, we recently proved that C-5 hydrogen of 5β-ketol **3** is retained completely during the subsequent conversion into **1**.[23] Thus, 30% of C-5 hydrogen of **1**, not derived from 6β-H of **6**, should be introduced in the step of *cis*-A,B-ring formation, i.e. **5**→**3**.

Although 7-dehydrocholesterol (**4**) is accepted as an obligatory intermediate in ecdysteroid biosynthesis in insects, no feeding studies of **4** have been reported in plants. Thus, [4-^{13}C]-7-dehydrocholesterol[24] was fed to *Ajuga* hairy roots to see whether it is metabolized into **1** or not. Figure 2 shows the ^{13}C-NMR spectrum of the resulting **1**, indicating incorporation of **4** into **1**. Rather poor incorporation of **4** into **1**, compared to that found in cholesterol, may be attributed to the instability of **4** in the incubation medium.

In conclusion, the present studies have provided several lines of evidence on the early steps of ecdysteroid biosynthesis in *Ajuga* hairy roots: (1) lathosterol was incorporated into 20-hydroxyecdysone. (2) Ca 70% of 6β-hydrogen of lathosterol migrated to the C-5 position of 20-hydroxyecdysone, whereas the remaining (ca 30%) of C-5 hydrogen of 20-hydroxyecdysone originated from another hydrogen source. This observation is very similar to that found in C-6 hydrogen of cholesterol. (3) 7-Dehydrocholesterol was shown to be converted into 20-hydroxycdysone in plants for the first time.

The present findings strongly suggested that the intermediacy of 7-dehydrocholesterol as well as 7-dehydrocholesterol 5α,6α-epoxide (Scheme 1). The partial (ca. 30%) loss of Hb (see Scheme 1) appears to be due to the instability of the epoxide, but a precise mechanism is an open question.

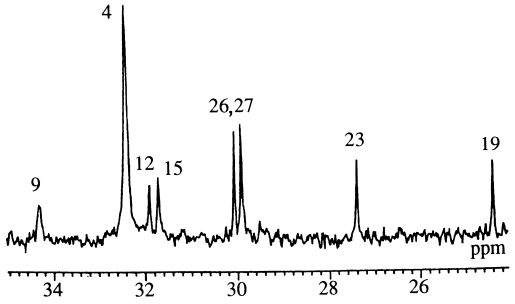


Figure 2. The ^{13}C-NMR spectrum (100 MHz, pyridine-d_5) of 20-hydroxyecdysone (**1**) derived from [4-^{13}C]-7-dehydrocholesterol.

Experimental

^{1}H NMR spectra were obtained on a JEOL EX-400 (400 MHz) or JEOL GSX-500 (500 MHz) spectrometer in $CDCl_3$ solutions and chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (used as internal reference). ^{13}C-NMR spectra were recorded on the same spectrometer (100 or 125 MHz) and chemical shifts are referenced to the $CDCl_3$ solvent (δ 77.0) or 2,5-C of pyridine-d_5 (δ 149.8). ^{2}H NMR spectra were obtained on a JEOL GSX-500 (77 MHz) spectrometer in a $CHCl_3$ or pyridine solution and chemical shifts (δ) are referenced to δ 7.26 for $CHCl_3$ or δ 7.19 for 2,5-H_2 of pyridine. HPLC were performed on Shimadzu LC-6A with SPD-6A UV detector using an ODS column.

[6-^{2}H]Cholesterol acetate (7). This compound, mp 114–116°C (lit.[25] 114–115°C), was prepared from 3β-tetrahydropyranyloxy-5α-cholestan-6-one[26] in four steps (reduction with $LiAlD_4$, dehydration with $POCl_3$/Py, deprotection of the THP group under an acidic condition, and acetylation with Ac_2O/Py).

[6-^{2}H]Cholesta-5,7-dien-3β-ol (8). *N*-bromosuccinimide (1.00 g, 5.62 mM) was added to a stirred solution of **7** (2.80 g, 6.53 mM) in dry CCl_4 (100 ml) under N_2 and the mixture was heated at reflux for 10 min and then cooled to 0°C. The resulting precipitate was filtered off and the filtrate was concentrated to dryness. The residue, dissolved in dry THF (15 ml), was treated with tetra-*N*-butylammonium bromide (29 mg, 0.091 mM). The mixture was stirred at room temperature for 45 min in the dark. Tetra-*N*-butyl-ammonium fluoride in THF (1 M solution, 20 ml) was added and the mixture was stirred for 15 min in the dark. AcOEt and water were added to the mixture and the separated organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed over silica gel with hexane:AcOEt (50:1) to give a crude diene (2.3 g). This was further purified on an $AgNO_3$ (20 g) impregnated silica gel (100 g) column hexane:CH_2Cl_2 (2:1) to give the diene acetate (1.3 g) as a yellow solid. This was dissolved in KOH/THF–MeOH (KOH 1.0 g, THF 25 ml, MeOH 75 ml) and stirred at room temperature for 1 h. Extractive (ether) workup and recrystallization of the residue from acetone afforded **8** (812 mg, 32%) as white crystals, mp 132–133°C (lit.[27] 143°C for unlabeled compound). ^{1}H NMR δ: 0.62 (3H, s, 18-H_3), 0.86 (6H, d, J=6.8 Hz, 26, 27-H_3), 0.94 (3H, d, J=6.2 Hz, 21-H_3), 0.94 (3H, s, 19-H_3), 3.63 (1H, m, 3-H), 5.38 (1H, brs, 7-H). Anal. Calcd. for $C_{27}H_{43}DO$: C, 84.10%; H+D, 11.50%. Found: C, 83.82%; H+D, 11.83%.

[6β-^{2}H]Lathosterol (6a). Raney nickel (W-2) (40 mg) was added to a solution of **8** (812 mg, 2.11 mM) in dry dioxane (11 ml) and the mixture was stirred for 2 days at room temperature under H_2. The catalyst was removed by filtration through a pad of Celite. The filtrate was washed with brine and the organic layer was dried over Na_2SO_4 and concentrated. The residue was recrystallized from 90% aqueous acetone to give **6a** (744 mg, 91%) as white crystals, mp 123–124°C (lit.[18] 122–122.5°C). ^{1}H NMR δ: 0.53 (3H, s, 18-H_3), 0.79 (3H, s, 19-H_3), 0.86 (6H, d, J=6.8 Hz, 26, 27-H_3), 0.92 (3H, d, J=6.8 Hz, 21-H3), 3.59 (1H, m, 3-H), 5.14 (1H, brd, J=4.0 Hz, 7-H). ^{2}H NMR δ: 1.74 (6β-^{2}H). ^{13}C-NMR δ: 29.24 (t, J=73 Hz, C-6). Anal. Calcd. for $C_{27}H_{45}DO$: C, 83.65%; H+D, 11.96%. Found: C, 83.60%; H+D, 12.26%.

[6β-^{2}H]-5α-Cholest-7-en-3-one (9). Molecular seives 4A (powder, 340 mg) and PCC (340 mg, 1.55 mM) were added to a solution of **6a** (300 mg, 0.775 mM) in dry CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature for 4 h. The mixture was diluted with dry ether and filtered through a Florisil pad. Concentration of the filtrate gave a crude ketone which was chromatographed over silica gel with hexane:AcOEt (30:1) to afford **14** (273 mg, 91%) as white crystals, mp 142–143°C (from acetone) (lit.[28] 143–145°C for unlabeled compound). ^{1}H NMR δ: 0.56 (3H, s, 18-H_3), 0.87 (6H, d, J=6.5 Hz, 26, 27-H_3), 0.93 (3H, d, J=6.2 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 5.18 (1H, brs, 7-H). Anal. Calcd. for $C_{27}H_{43}DO$: C, 84.09%; H+D, 11.50%. Found: C, 84.38%; H+D, 11.79%.

[3α,6β-$^2H_2$]Lathosterol (6b). $LiAlD_4$ (14 mg, 0.333 mM) was added to a stirred solution of **9** (254 mg, 0.660 mM) in dry ether (10 mL) at room temperature under N_2. The mixture was stirred for 30 min and then diluted with moist ether and dil. HCl. Extractive (AcOEt) work up and purification of the residue on a silica gel Lobar column with hexane:AcOEt (6:1) gave **6b** (196 mg, 71%) as a white solid, mp 124–125°C (from MeOH) (lit.[18] 120–123°C for unlabeled compound). ^{1}H NMR δ: 0.53 (3H, s, 18-H_3), 0.79 (3H, s, 19-H_3), 0.86 (6H, d, J=6.4 Hz, 26, 27-H_3), 0.92 (3H, d, J=6.4 Hz, 21-H_3), 5.16 (1H, brd, J=5.8 Hz, 7-H). ^{2}H NMR δ: 1.74 (6β-^{2}H), 3.58 (3-^{2}H). Anal. calcd for $C_{27}H_{44}D_2O$: C, 83.44%; H+D, 11.93%. Found: C, 83.53%; H+D, 12.04%.

[6α-^{2}H]-7α-Bromo-5α-cholestane-3β,6β-diol (11). $LiAlD_4$ (1.00 g, 9.07 mM) was added to a stirred solution of 3β-acetoxy-7α-bromo-5α-cholestan-6-one **10**[18] (2.80 g, 5.32 mM) in dry ether (30 mL) at 0°C under N_2 and the mixture was stirred for 1.5 h. The mixture was worked up in a manner similar to that described for **6b** [Lobar column was eluted with $CHCl_3$:AcOEt (10:1)] to afford **11** (894 mg, 34%), mp 95–97°C. ^{1}H NMR δ: 0.71 (3H, s, 18-H_3), 0.87 (6H, d, J=5.9 Hz, 26, 27-H_3), 0.91 (3H, d, J=6.8 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.71 (1H, m, 3-H), 3.92 (1H, brs, 6-H), 4.24 (1H, brs, 7-H). Anal. Calcd. for $C_{27}H_{46}DBrO_2$: C, 66.53%; H+D, 10.81%. Found: C, 66.92%; H+D, 9.78%.

[6-^{2}H]-3β-Acetoxy-5α-cholest-6-ene (12). Zn powder (1.80 g, 334 mM) was added to a solution of **11** (894 mg, 1.85 mM) in acetic acid (20 mL) and the mixture was heated at reflux for 1.5 h under N_2. The mixture was cooled to room temperature and diluted with water and AcOEt. Extractive (AcOEt) work up gave 6-en-3β-ol (696 mg) as a white solid. This was treated with pyridine and acetic anhydride to give a crude acetate which was chromatographed over silica gel hexane:AcOEt (50:1).

Recrystallization of the residue from MeOH gave **12** (644 mg, 81%) as white crystals, mp 108–109°C (lit.[19] 105.8–106.6°C for unlabeled compound), ^{1}H NMR δ: 0.69 (3H, s, 18-H_3), 0.86 (6H, d, J = 6.5 Hz, 26, 27-H_3), 0.91 (3H, d, J = 6.5 Hz, 21-H_3), 0.79 (3H, s, 19-H_3), 2.03 (3H, s, Ac), 4.73 (1H, m, 3-H), 5.48 (1H, brs, 7-H). Anal. Calcd. for $C_{29}H_{47}DO_2$: C, 81.06%; H + D, 11.26%. Found: C, 80.93%; H + D, 11.54%.

[6β-^{2}H]-3β-Acetoxy-6α,7α-epoxy-5α-cholestane (13). A solution of **12** (612 mg, 1.43 mM) in dry CH_2Cl_2 (10 mL) was treated with mCPBA (492 mg, 2.85 mM) and the mixture was stirred at room temperature for 1 h under N_2. Addition of sat. aq $NaHCO_3$ and extractive (AcOEt) workup gave a crude product which was chromatographed over silica gel column hexane:AcOEt (10:1) to give **13** (629 mg, 99%) as a white solid, mp 180–181°C (lit.[18] 176.5–177.8°C for unlabeled compound). ^{1}H NMR δ: 0.71 (3H, s, 18-H_3), 0.87 (6H, d, J = 6.5 Hz, 26, 27-H_3), 0.90 (3H, d, J = 6.8 Hz, 21-H_3), 0.79 (3H, s, 19-H_3), 2.04 (3H, s, Ac), 3.02 (1H, d, J = 2.2 Hz, 7-H), 4.74 (1H, m, 3-H). Anal. Calcd. for $C_{29}H_{47}DO_3$: C, 78.15%; H + D, 10.85%. Found: C, 78.27%; H + D, 11.14%.

[6α-^{2}H]-5α-Cholestane-3β,7α-diol (14). Reduction of **13** (597 mg, 1.34 mM) with $LiAlH_4$ (357 mg, 9.39 mM) and workup in a manner similar to that described for **6b** gave, after concentration of the extract, **14** (600 mg, quant.) as a white solid, mp 156–157°C (from MeOH) (lit.[29] 151–152°C for unlabeled compound). ^{1}H NMR δ: 0.66 (3H, s, 18-H_3), 0.86 (6H, d, J = 6.5 Hz, 26, 27-H_3), 0.91 (3H, d, J = 6.2 Hz, 21-H_3), 0.81 (3H, s, 19-H_3), 3.63 (1H, m, 3-H), 3.83 (1H, brs, 7-H). Anal. Calcd. for $C_{27}H_{45}DO_2$: C, 79.94%; H + D, 11.93%. Found: C, 80.18%; H + D, 12.23%.

[6α-^{2}H]-5α-Cholestane-3β,7α-diol 3-benzoate (15). BzCl (332 μL, 2.83 mM) was added to a stirred solution of **14** (574 mg, 1.42 mM) in pyridine (5 mL) at room temperature under N_2. The mixture was reacted at room temperature for 2 h. Ice chips were added and the mixture was stirred for 10 min. Extractive (AcOEt) workup gave a crude product which was chromatographed over silica gel with hexane:AcOEt (10:1) to afford **15** (526 mg, 73%) as a white solid, mp 163–165°C (from MeOH). ^{1}H NMR δ: 0.67 (3H, s, 18-H_3), 0.87 (6H, d, J = 6.5 Hz, 26, 27–H_3), 0.91 (3H, d, J = 6.5 Hz, 21-H_3), 0.88 (3H, s, 19-H_3), 3.84 (1H, brs, 7-H), 4.97 (1H, m, 3-H), 7.4–8.1 (5H, m, aromatic). Anal. Calcd. for $C_{34}H_{51}DO_3$: C, 80.11%; H + D, 10.28%. Found: C, 80.39%; H + D, 10.27%.

[6α-^{2}H]Lathosterol (16). $POCl_3$ (174 μL, 1.87 mM) was added to a solution of **15** (640 mg, 1.26 mM) in pyridine (6.0 mL) at room temperature under N_2 and the mixture was stirred overnight. Water and AcOEt were added to the mixture and extractive (AcOEt) work up gave 7-ene (573 mg). This was treated with 10% KOH–MeOH (3 mL) and THF (6 mL) at room temperature for 1 h. Extractive (AcOEt) work up and purification of the residue over silica gel with hexane:AcOEt (9:1) afforded **16** (183 mg, 38%) as a white solid, mp 123–125°C (from MeOH) (lit.[18] 120–123°C for unlabeled compound). ^{1}H NMR δ: 0.53 (3H, s, 18-H_3), 0.86 (6H, d, J = 6.6 Hz, 26, 27-H_3), 0.92 (3H, d, J = 6.6 Hz, 21-H_3), 0.80 (3H, s, 19-H_3), 3.59 (1H, m, 3-H), 5.15 (1H, brs, 7-H). ^{2}H NMR δ: 1.77 (6α-^{2}H). Anal. Calcd. for $C_{27}H_{45}DO$: C, 83.65%; H + D, 11.50%. Found: C, 83.50%; H + D, 12.24%.

[6α-^{2}H]-5α-Cholest-7-en-3-one (17). Compound **16** (336 mg, 0.87 mM) was oxidized in a manner similar to that described for **9** to afford **17** (238 mg, 71%) as a white solid, mp 141–142°C (from acetone). (lit.[27] 143–145°C for unlabeled compound). ^{1}H NMR δ: 0.56 (3H, s, 18-H_3), 0.87 (6H, d, J = 6.6 Hz, 26, 27-H_3), 0.93 (3H, d, J = 6.5 Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 5.18 (1H, brs, 7-H). Anal. Calcd. for $C_{27}H_{43}DO$: C, 84.09%; H + D, 11.50%. Found: C, 84.33%; H + D, 11.73%.

[3α,6α-^{2}H$_2$]Lathosterol (6c). Compound **17** (227 mg, 0.59 mM) was reduced with $LiAlD_4$ (12 mg, 0.29 mM) in a manner similar to that described for **11** to give **6c** (206 mg, 86%) as a white solid, mp 124–125°C (from MeOH). ^{1}H NMR δ 0.53 (3H, s, 18-H_3), 0.86 (6H, d, J = 6.8 Hz, 26, 27-H_3), 0.92 (3H, d, J = 6.5 Hz, 21-H_3), 0.79 (3H, s, 19-H_3), 5.15 (1H, brs, 7-H). Anal. calcd for $C_{27}H_{44}D_2O$: C, 83.44%; H + D, 11.93%. Found: C, 83.39%; H, 12.23%.

Incubation with *Ajuga* hairy roots. *Ajuga* hairy roots were maintained as described previously.[6] To pre-incubated cultures (four 500-mL flasks, each containing 250 mL of MS medium) were added the labeled sterols (100 mg each), **6a**, **6b** or **6c**, dissolved in acetone (4 mL) and Tween 80 (2 mL) as described previously.[5] The roots, weighing ca 110 g (wet wt), were extracted and separated to furnish 4.2, 2.1 and 2.8 mg of **1** from **6a**, **6b** and **6c**, respectively, as described previously.[5] The sterol fraction was obtained from the fractions eluted with $CHCl_3$:MeOH (10:1–7:1) and chromatographed again over silica gel with hexane-AcOEt (3:1) to yield a purified sterol fraction. A portion of the sterol fraction was further purified by HPLC to furnish cholesterol (column, Shimadzu Shimpack CLC-ODS; 15×6 mm i.d.; solvent, MeOH; flow rate, 1.0 ml/min; detection at 210 nm, retention time 16.6 min).

[4-^{13}C]-7-Dehydrocholesterol (41 mg) was fed to the hairy roots in a similar manner to give 1.1 mg of 20-hydroxyecdysone.

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