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SYNTHESIS OF [14α-METHYL-^{3}H]-24,25-DIHYDROLANOSTEROL*

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SUMMARY

We describe, herein, the first synthesis of isomerically pure title compound (**6**) at high specific activity. This required the development of a convenient, regiospecific synthesis of the $\Delta^{8(9)}$-15-ketone (**15**) and subsequent alkylation with methyl-[$^3H_3$] iodide. A key step in our procedure was the use of an *electrochemical* reduction of the intermediate [14α-methyl-^{3}H]-15-oxo-dihydrolanosterol (**19**). This process was effected cleanly and in high yield to give (**6**), an important assay tool in the search for cholesterol lowering agents. This approach was found to be significantly superior to the Wolff-Kishner reduction of the corresponding 3-benzoate (**18**).

Key Words: Electrochemical reduction, dihydrolanosterol-^{3}H, lanosterol demethylase

INTRODUCTION

The biochemical conversion of lanosterol (**1**) to cholesterol requires the removal of three methyl groups, isomerization of the 8(9)-double bond to the 5(6)-position, and reduction of the 24(25)-double bond. The initial and rate limiting event in this cascade is the oxidative removal of the 14α-methyl group (1), a process which is mediated by the microsomal enzyme lanosterol 14α-demethylase (2). A substance which inhibited this key enzyme would, therefore, have potential value as a cholesterol lowering agent. The availability of [14α-methyl-^{3}H]-24,25-dihydrolanosterol (DHL) (**6**), which is an effective substrate for the

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enzyme, greatly enhances the sensitivity and efficiency of assays designed to screen for 14α-demethylase inhibitors. We report herein conditions for the synthesis of (6) which provide regiochemically pure material of high specific activity, and in good yield.

DISCUSSION

It is known (3) that 14α-demethylation of lanosterol (1) occurs by a series of oxidations performed by a single enzyme (as shown in **figure 1**), in which the 14α-methyl is converted sequentially to give the hydroxymethyl compound (2), the formyl derivative (3), and finally the diene (4). The aldehyde function is lost as formic acid, and not as CO_2 (3).

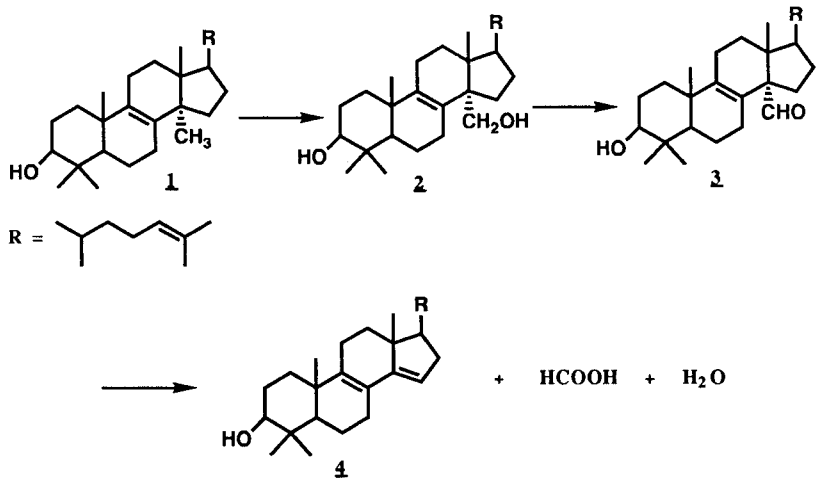
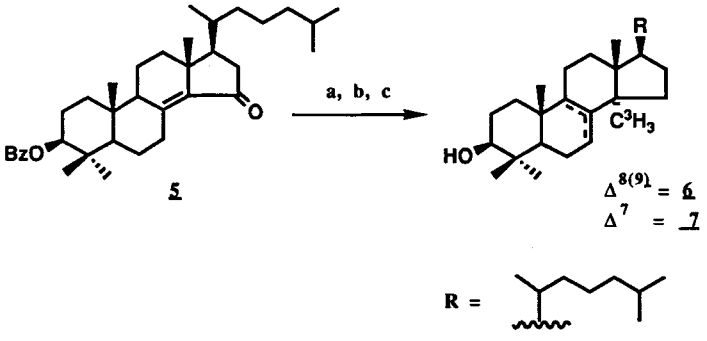


figure 1: biochemical 14α-demethylation of lanosterol mediated by lanosterol 14α-demethylase

Thus, if the 14α-methyl group of (1) (or a close analog) were labelled with tritium, water soluble, tritiated by-products (formic acid and water) would be released into the system. The effectiveness of inhibitors of the lanosterol demethylase enzyme could then be quantitated by using such a reagent in competitive experiments, and measuring of the amount of tritium in the aqueous phase after removal of lipophilic products by extraction. Such an assay has, in fact, been developed (4) and found to be both more efficient and more sensitive than earlier GC (5) or HPLC (6) based assays.

The [14α-methyl-^{3}H]-DHL (6) used in the reported work was prepared from $\Delta^{8(14)}$-15-oxo-DHL (5) *via* formation of a silyl enol ether and alkylation with low specific activity [^{3}H]CH_3I (4) (see **scheme 1**). Wolff-Kishner reduction of the intermediate ketone then gave desired product (6) at a

specific activity of 350mCi/mmol and in 0.3% overall yield. It is noteworthy that the product (6) prepared in this manner contained 12% of the corresponding Δ^7-olefin isomer (7), which is apparently an unavoidable consequence of using the reasonably accessible $\Delta^{8(14)}$-ketone (5) as the key intermediate (7). Tritium labelled 32-hydroxy-DHL was also prepared and used in the cited work (4), but this compound is of less interest since its use in an assay would result in loss of sensitivity in the first step of the demethylase cascade.

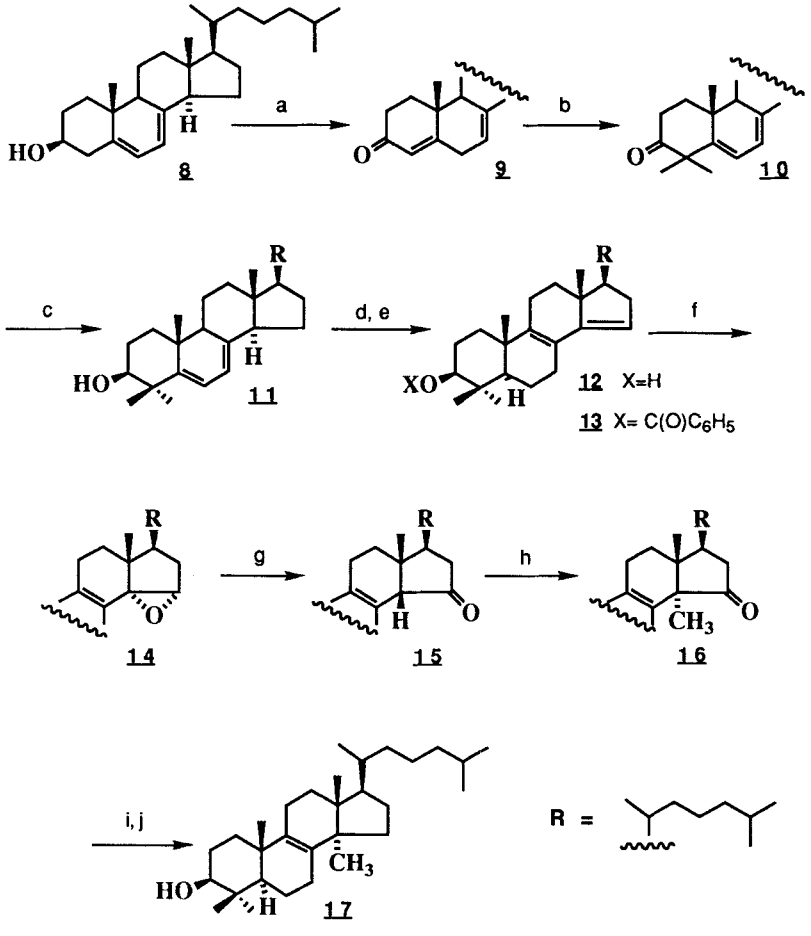


scheme 1: synthesis of 6 from the $\Delta^{8(14)}$-15-ketone 5 (4)
a: $(CH_3)_3Si$ Cl, TEA ; b: [^{3}H]CH_3I, TBAF ; c: Wolff-Kishner reduction

In terms of acceptable modifications to the lanosterol structure for ease of synthesis, the presence or absence of the side-chain Δ^{24}-olefin does not appear to significantly affect metabolism by the 14-demethylase enzyme (14-LDM) (8). However, the Δ^7-olefin is known to be a considerably poorer substrate for the enzyme than the natural Δ^8-isomer (8). The purpose of our work, therefore, became the preparation of isomerically pure [14α-methyl-^{3}H]-Δ^8-DHL (6) of high specific activity. The use of this material should provide the optimum accuracy and sensitivity in a screening assay for lanosterol demethylase inhibitors.

At the time this work was initiated, it was not obvious how to prepare isomerically pure DHL (17) by a route wherein the 14-methyl group could be put in last, in a straightforward manner and in a reasonable yield (9). We planned to proceed via the $\Delta^{8(9),14}$-diene (12) to prepare $\Delta^{8(9)}$-15-ketone (15) as the key intermediate for our route, and then to work out the alkylation at C-14 with unlabelled methyl iodide before using tritiated material. An attractive route to dienes such as (12) is through the acid-catalyzed rearrangement of sterol $\Delta^{5,7}$-dienes, but this procedure has been plagued by the production of double-bond isomers which are difficult to separate (10). This stumbling block to the preparation of pure Δ^8-sterols was recently overcome by Dolle and Kruse in the cholestane series, where modified conditions for the acid-catalyzed rearrangement gave clean conversions of $\Delta^{5,7}$-dienes into the corresponding $\Delta^{8(9),14}$-dienes as single products (11). It was not clear how this rearrangement

would apply in the corresponding lanostane series. Thus, the present work was begun by transforming 7-dehydrocholesterol (**8**) sequentially *via* an Oppenauer oxidation (12) into ketone (**9**), alkylation with t-BuOK/t-BuOH and CH_3I to give 4,4-dimethyl ketone (**10**), and reduction with $LiAlH_4$ to give $\Delta^{5,7}$-diene-3β-alcohol (**11**) (**scheme 2**). Rearrangement of the diene system was performed using a saturated solution of HCl gas in a 5:1 $CHCl_3$/HOAc mixture at 10°. Treatment of (**11**) with this system followed by reflux in 5:1 $CHCl_3$/HOAc did, indeed, result in clean conversion of (**11**) to the desired $\Delta^{8(9),14}$-diene (**12**), in 64.5% isolated yield. Further reaction with benzoyl chloride/pyridine gave the 3β-benzoate (**13**) (98%), a 29% overall yield from (**8**).

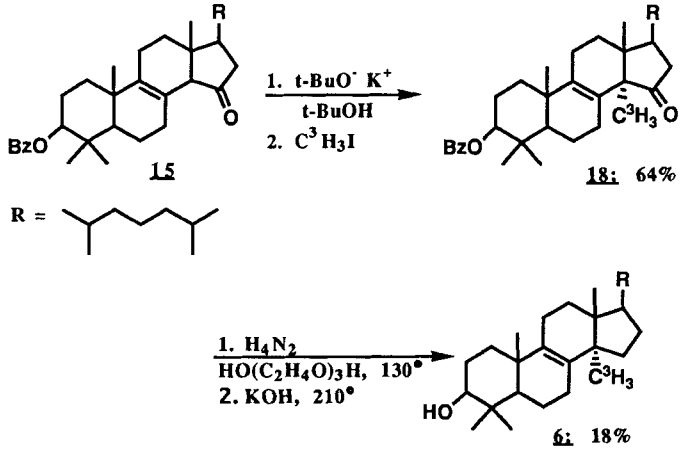


scheme 2: synthesis of intermediate ketone 15 and of cold DHL 17

a: $Al(Oi\text{-}Pr)_3$, cyclohexane ; b: t-BuOK, CH_3I ; c: $LiAlH_4$; d : HCl in $CHCl_3$/HOAc; e:C_6H_5COCl/pyridine ; f: m-CPBA ; g: BF_3-Et_2O ; h: t-BuOK, CH_3I ; i: N_2H_4-HCl, N_2H_4, $HO(C_2H_4O)_3H$, 130°; j: KOH, 210°.

Oxidation of $\Delta^{8,(9),14}$-diene (**13**) with 80-85% m-chloro-perbenzoic acid gave the labile epoxide (**14**), which was used without isolation (13). Rearrangement of (**14**) in the presence of $BF_3.Et_2O$ then gave desired $\Delta^{8(9)}$-15-ketone (**15**) in 38.7% overall yield from (**13**). Treatment of (**15**) with 1.5 eq. of t-BuOK in t-BuOH for 10 min followed by addition of 4 eq. of CH_3I and reaction for 40 min at 20°, then afforded 14α-methyl ketone (**16**) as the sole product of thermodynamic alkylation (14). Pure (**16**) was isolated in 56% yield by recrystallization of the crude product from CH_2Cl_2-CH_3OH, which removed all the unreacted starting enone (**15**). This was fortunate, since (**15**) and (**16**) could not be separated by chromatography. Wolff-Kishner reduction (15) of (**16**) then afforded authentic 24,25-DHL (**17**) in 72% yield [28% from enone (**15**), and 6.6% overall from (**8**)]. There was no indication of the presence of double bond isomers in the nmr spectra of (**15**), (**16**), or (**17**), so that this route could be relied on to provide pure Δ^8-compounds. The intermediate enone (**15**) was then used to prepared the [14α-Me-^{3}H]-compound (**6**).

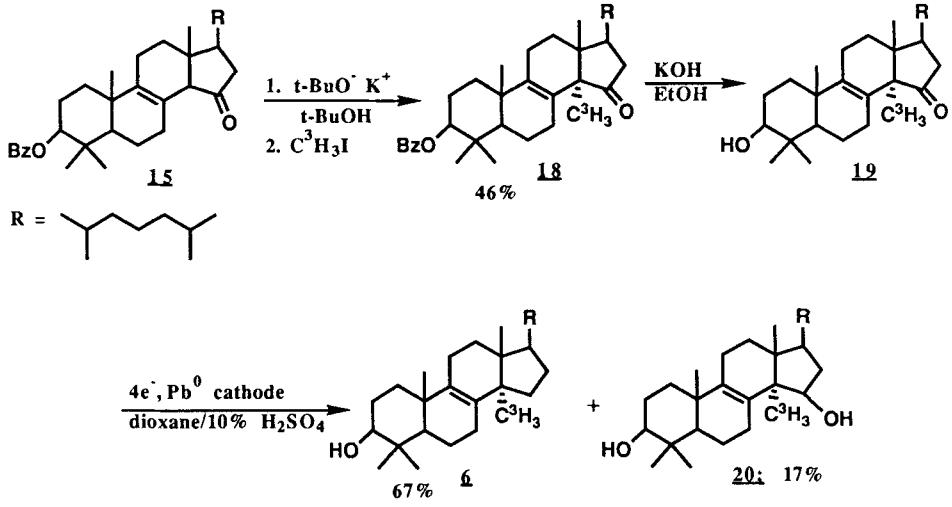
Our first attempt to synthesize (**6**) by substituting C^3H_3I for CH_3I in the penultimate step of the above route resulted in an overall 11.7% yield of pure Δ^8-material from enone (**15**) (see **scheme 3**). This was a significant improvement over the previously reported preparation of (**6**) (in 0.3% yield) (4). Thus, the enolate of (**15**) was generated with t-BuOK/t-BuOH and alkylated with slightly less than one equivalent of C^3H_3I at @ 200 mCi/mmole, giving a mixture of labelled methyl ketone (**18**) with unreacted (**15**). Crystallization of the crude product from methanol-dichloromethane then furnished pure labelled 14α-methyl ketone (**18**) in 64% yield. Subsequent Wolff-Kishner reduction of 31 mCi of (**18**) gave (**6**) in what appeared to be a very high yield (at least 85%) by TLC analysis (silica gel, 10% ethyl acetate-hexane). However, column chromatography



scheme 3: synthesis of 14α-methyl-[^{3}H]-DHL via Wolff-Kishner reduction

using the same system afforded only 7.5 mCi of (6) which was still contaminated with a small labelled and a significant unlabelled impurity. Final purification was effected by preparative reverse phase HPLC (see experimental). A total of 5.5 mCi of pure (6) was thus obtained at a specific activity 182 mCi/mmole.

Although the product obtained in this synthesis was adequate for initial screening of potential 14α-demethylase inhibitors, higher specific activity material was soon required in order to increase the sensitivity of the assay. Our second synthesis is shown in **scheme 4**. Commercially available C^3H_3I (250 mCi) at 85 Ci/mmole was diluted on a vacuum line with carrier to a specific activity of approximately 8 Ci/mmole. The enolate of (15) was then generated and methylated as described above to furnish (18) in 46% yield. There is no obvious reason for the lower yield (compared to the previous synthesis) except for the 40 fold decrease in scale. We have found that some reactions afford lower than expected yields when micro-scale conditions are employed. This may be due to the attenuation of reaction rates caused by higher than normal dilution and/or the fact that it is often difficult to control stoichiometry at these levels.



scheme 4: synthesis of 14α-methyl-[^{3}H]-DHL via electrochemical reduction

Having encountered poor isolated yields of (6) via the Wolff-Kishner process, we thought it would be useful to explore alternate approaches. It occurred to us that the electrochemical reduction of steroidal ketones to methylenes, which had been reported to proceed in high yield (16), would be particularly well suited to micro-scale radiochemical reactions because of the mildness of the conditions. Furthermore, the specificity of electrochemical processes would be expected to furnish clean reaction products.

Exploratory experiments with unalbelled substrate showed that a free 3-hydroxyl was essential in order to achieve sufficient solubility in the electrolysis medium. To this end, benzoate (**18**) was saponified in ethanolic KOH to give alcohol (**19**) in 91% yield. After successfully evaluating this process with the unlabelled analog of (**19**), we proceeded with the labelled reaction. The cathode compartment of a standard electrolysis cell equipped with a lead foil cathode and a carbon anode was charged with a solution of (**18**) in dioxane-10% sulfuric acid (1:1). The anode compartment was charged with the same electrolyte solution and an initial current of 400 mA was applied and increased incrementally to 1200 mA over a 12 hr period. The progress of the reaction was monitored by TLC (20% ethyl acetate-hexane). The ratio of product to starting material did not increase in a regular fashion. This was due to the fact that, even at a concentration of 0.5 mg/35 mL, the product (**6**) was quite insoluble in the electrolysis medium and tended to collect on the walls of the cell and on the thermometer. The electrolysis was simply continued until the amount of starting material was negligible. The contents of the cathode compartment were then extracted with ether and the compartment walls, thermometer, and stirring magnet were rinsed with ether. The combined ether solutions were washed with $NaHCO_3$ and dried over Na_2SO_4. Purification on the Chromatotron (silica gel, 5-30% ethyl acetate-hexane gradient) afforded 54 mCi (67%) of pure [14α-methyl-$^3H_3$]-DHL (**6**). The specific activity was determined by mass spectroscopy to be 8.3 Ci/mmol. The usual specific activity determination using UV and radioassay was avoided because of the low extinction coefficient of the product. [14α-Methyl-$^3H_3$]-15-hydroxy-DHL (**20**) was obtained in 17% yield as a by-product of the electrolytic reduction.

The synthetic sequence described above represents the first cohesive description of a route to the 15-ketone (**15**). This key intermediate allowed straightforward access to *isomerically pure* [14α-methyl-^{3}H]-DHL (**6**) at high specific activity. The availibility of this material provides an improved and highly sensitive assay tool for the investigation of inhibitors of the lanosterol demethylase enzyme. In addition, the mild conditions, clean reaction mixtures, and high yields observed in the reduction of the 15-oxo-DHL-^{3}H (**19**) to (**6**) should make electrochemical processes an especially useful addition to the radiochemist's repertoire.

Acknowledgements: We wish to thank Dr. Gary Lee for his advice and many helpful discussions regarding the electrochemistry work. Thanks are also due to Dr. K. Walker for helpful discussions related to the synthesis of ketone (**15**).

EXPERIMENTAL

Unlabelled reagents were purchased from Aldrich Chemical Co. and used without purification. Methyl-[^{3}H] iodide was purchased from American

Radiolabelled Chemicals. Solvents were HPLC grade. Electrolytic reductions were performed using equipment manufactured by the Electrosynthesis Co., E. Amherst, New York. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. UV spectra were obtained using a Hitachi UV-265 spectrophotometer. Nmr spectra were generated on a Brucker ACF 300 in $CDCl_3$ solution using TMS as an internal standard. Mass spectra were recorded on a Finnigan MAT 3280. The nmr and mass spectra, and the elemental analyses of all unlabelled reaction products were consistent with their proposed structures.

Cholesta-4,7-dien-3-one (9)

A mixture of 7-dehydrocholesterol (**8**) (25.5g, 66.3 mmol), cyclohexanone (86mL, 830 mmol) and toluene (330mL) was heated at reflux in a 1L flask equipped with mechanical stirrer, Dean-Stark trap, and condenser. After water had ceased to collect in the trap (2h), the mixture was cooled to 80° and aluminum isopropoxide (7.5g, 36.7 mmol) was added all at once. The mixture was heated at reflux again for 1h, after which it was cooled in ice and then poured into 2N aqueous HCl (300mL). The organic layer was washed with 2N HCl, water, and dried over Na_2SO_4, prior to removal of solvents under reduced pressure. The residue was further concentrated *in vacuo* giving 28.2g of a yellow syrup. Flash chromatography (7% acetone in hexane) then gave essentially pure (**9**) (21.7g, 86%) as an off-white crystalline solid, mp 68-70°; mass spectrum ($C_{27}H_{42}O$), *m/e* 382 (M+).

4,4-Dimethylcholesta-5,7-dien-3-one (10)

To a solution of t-BuOK (25.5g, 227 mmol) in t-BuOH (550mL) at 45° was added a solution of (**9**) (21.7g, 56.7 mmol) in THF (50mL). After 10 min, methyl iodide (28.2mL, 455 mmol) was added and the mixture was warmed at 50° for another 45 min, after which it was concentrated to approximately 200mL by distillation under vacuum. The residue was diluted with EtOAc (800mL) and water (800mL). The precipitate which formed was filtered, washed with EtOAc and water, then dried *in vacuo*, affording 11.5g of pure (**10**). The EtOAc filtrate and washes were combined, further washed with water, dried over Na_2SO_4, reduced *in vacuo* to a volume of 200mL, and cooled in ice. The resulting precipitate was filtered and dried, giving another 7.1g of nearly pure (**10**) as an off-white solid, in a total yield of 18.6g (78%). The 11.5g first crop of (**10**) was a white solid of analytical quality; mp 155-158°; mass spectrum ($C_{29}H_{46}O$), *m/e* 410 (M+).

4,4-Dimethylcholesta-5,7-dien-3α-ol (11)

To a mechanically stirred slurry of $LiAlH_4$ (40g, 26.4mmol) in THF (50mL) at room temperature was added a solution of (**10**) (18.4g, 44.8mmol) in THF (400mL) dropwise over 1 h, and stirring was continued. After 1h the reaction was noted to be incomplete by TLC, and another portion of $LiAlH_4$ (1.0g, 26.4 mmol) was added. After another 1h of stirring, excess $LiAlH_4$

was destroyed by adding a 1:1 mixture of H_2O-THF. The solution was dried over $MgSO_4$ and filtered through celite. The solvent was removed by distillation *in vacuo* affording nearly pure (**11**) (18.4g, 99%): mp 130-132°; mass spectrum ($C_{29}H_{48}O$), *m/e* 412 (M+).

4,4-Dimethylcholesta-8,14-dien-3β-ol (12)

A 5:1 mixture of chloroform (300mL) and acetic acid (60mL) was prepared. A portion of the mixture (100mL) was cooled in a mixing cylinder and held at 6-10° while dry HCl gas was bubbled through for 15 min to saturation. The HCl solution was then added dropwise over 20 min to a solution of (**11**) (18.2g, 44.1 mmol) in the 5:1 solvent mixture (240mL) at 10°, after which the mixture was warmed to room temperature over 20 min and then heated at reflux for 30 min. The TLC of the mixture (15% EtOAc-hexane) showed that a clean transformation of (**11**) to a slightly less polar product had occurred. Solvents were removed by distillation *in vacuo* at under 50° and the resulting residue was recrystallized from MeOH-CH_2Cl_2, affording (**12**) (11.7g, 65%) of analytical purity : mp 133-135°; mass spectrum ($C_{29}H_{48}O$), *m/e* 412 (M+).

4,4-Dimethylcholesta-8,14-dien-3β-ol Benzoate (13)

A solution of (**12**) (11.7g, 28.4 mmol) in pyridine (125mL) was cooled to 10° and benzoyl chloride (7.0mL, 60.3 mmol) was added over 5 min. The mixture was allowed to warm and stir at room temperature for 24h, after which it was poured into ice-water (400mL). The resulting precipitate was filtered, washed with water, and dried *in vacuo* affording nearly pure (**13**) (14.3g, 98%): mp 152-154°; mass spectrum ($C_{36}H_{52}O_2$) *m/e* 516 (M+).

(14β)-4,4-Dimethylcholest-8-en-3β-ol-15-one Benzoate (15)

To a solution of (**13**) (4.13g, 8.0 mmol) in ethyl ether (110mL) at 0° was added a solution of meta-chloroperbenzoic acid (m-CPBA) (3.18g, 18.4 mmol) in ethyl ether (20mL) dropwise over 20 min. After 45 min, more m-CPBA (0.5g, 2.9mm) was added, and after another 15 min at 0°, dimethyl sulfide (2mL) was added. The solution was washed sequentially with dilute NH_4OH, water, and brine, then dried over Na_2SO_4. The solvent was changed by adding benzene (100mL) and removing the ether by distillation *in vacuo* at under 40°.

The resulting benzene solution of epoxide (**14**) was cooled to 10° and boron trifluoride etherate (BF_3•Et_2O), 1.2mL, 9.4 mmol) was added. After 15 min, another portion of BF_3•Et_2O (0.85mL, 6.7 mmol) was added. After 1h, the mixture was diluted with EtOAc, washed with water, dried over Na_2SO_4, and concentrated to dryness. Crystallization of the residue from acetone-hexane furnished analytically pure (**15**) (1.26g), and flash chromatography (6% EtOAc-hexane) of the mother liquor materials followed by recrystallization gave another portion of (**15**) (0.39g), thus affording a total of 1.65g; 39%). The first isolated material gave physical data as follows: mp 153-155° (Lit. (14,17) mp-154-155°); mass spectrum ($C_{36}H_{52}O_3$), *m/e* 532 (M+).

Lanost-8-en-3β-ol-15-one Benzoate (16)

To a solution of potassium t-BuOK (23mg, 0.19 mmol) in t-BuOH (3mL) was added a solution of (**15**) (80mg, 0.15 mmol) in THF (1mL). After 5 min, methyl iodide (38μL, 0.61 mmol) was added and stirring was continued for 1h. The mixture was diluted with EtOAc, washed with aqueous NH_4Cl followed by water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from CH_3OH-CH_2Cl_2, giving pure (**16**) (43mg, 56%) as a white solid: mp 203-205° (Lit. (14) mp 199-200°); mass spectrum ($C_{37}H_{54}O_3$), *m/e* 546 (M+).

24,25-Dihydrolanosterol (17)

A mixture of ketone (**16**) (60mg, 0.11 mmol), hydrazine hydrochloride (515mg, 7.5 mmol), triethylene glycol (5mL), and anhydrous hydrazine (2.8mL) was stirred and heated under argon in a 135° bath for 12h. The resulting homogeneous solution was cooled somewhat, potassium hydroxide (830mg, 15mmol) was added, and the reaction flask was refitted with a short-path distillation apparatus. The mixture, under a stream of argon, was then heated gradually to 210° over 30 min to distill off the hydrazine and was maintained at 210° for another 2h. The reaction was cooled and the resulting gel was dissolved by shaking with ethyl ether and water. The organic layer was separated, washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. Recrystallization of the residue from CH_3OH-CH_2Cl_2 gave essentially pure (**17**) (34mg, 72%). A portion of (**17**) was recrystallized (CH_3OH-CH_2Cl_2) for analysis : mp 141-142° (Lit. (18) mp 147°); mass spectrum ($C_{30}H_{52}O$), *m/e* 428 (M+).

[14α-Methyl-^{3}H]-15-oxo-24,25-dihydrolanosterol (19)

A 5 mL stopcock flask was charged with 16.3 mg (0.03 mmole) of 15-oxo-3-benzoate (**15**). The flask was connected to a vacuum line and evacuated. THF (0.3 mL) over $LiAlH_4$ was vacuum transferred into the reaction flask. The system was brought to atmospheric pressure with dry N_2 and a solution (40 μL, 1.02N, 0.04 mmole) of t-BuOK in THF was added (with a micro pipet) to the stirred solution of ketone at ambient temperature. After 20 min a one drop aliquot was quenched with an excess of unlabelled MeI. Analysis of this quench by HPLC confirmed the formation of enolate.

[^{3}H]MeI (250 mCi, 85 Ci/mmole, 0.003mmole) was diluted with unlabelled MeI (80 μL of a 0.32M solution in THF, 0.026 mmole) on the vacuum line to generate [^{3}H]MeI having a specific activity of about 8.6 Ci/mmole (0.029 mmole).

The [^{3}H]MeI was vacuum transferred into the enolate solution which had been frozen in liquid N_2. The system was warmed to ambient and stirred for 30 min. HPLC analysis of a reaction aliquot indicated the complete absence of starting material. Volatiles were removed by vacuum transfer into a solution of KOH in ethanol at -78° and the residue was quenched

with aqueous NH_4Cl. The crude product was isolated by four extractions with EtOAc. The combined organic phases (150 mCi) were washed with brine and dried over Na_2SO_4. Purification by Chromatotron chromatography (2 mm silica gel rotor, 0-10% EtOAc-hexane gradient) afforded 93 mCi of pure [14α-Me-^{3}H]-15-oxo-dihydrolanosterol-3-benzoate (**18**) plus 23 mCi of mixed fractions containing (**18**) and (**19**). Hydrolysis of pure (**18**) with ethanolic KOH followed by evaporation of the solvent and aqueous workup gave 84 mCi (90% yield) of pure (radio-TLC: silica gel, 20% EtOAc-hexane) title compound (**19**). In a synthesis identical to the above except for the use of [^{3}H]MeI having a specific activity of about 200 mCi/mmole, compound (**19**) was obtained in 64% yield.

[14α-Methyl-^{3}H]-24,25-dihydrolanosterol (6), Wolff-Kishner reduction

The low specific activity benzoate (**18**) (77 mg, 0.146 mmole, 31.5 mCi, 215 mCi/mmole) described above was combined with hydrazine hydrochloride (684 mg, 10 mmole), anhydrous hydrazine (3.6 mL) and triethylene glycol (6.4 mL). The reaction was heated overnight at 138° under a nitrogen atmosphere. KOH pellets (971 mg, 17 mmole) were added and the temperature was increased to 210° while the volatile reaction components were removed by distillation. After cooling to ambient temperature, the reaction mixture was partitioned between ethyl acetate and water. The organic phase was washed sequentially with dilute HCl, bicarbonate, water, and brine, then dried over sodium sulfate. The organic extract contained 25 mCi. Purification by column chromatography (10% EtOAc-hexane), Chromatotron (hexane), and finally reverse phase HPLC (Vydac 218TP1010, 0.5 in x 25 cm, 30% i-PrOH-CH_3CN, 5 mL/min) afforded 5.1 mCi (16.2 % yield) of pure (**6**). The specific activity was determined by weight to be 182 mCi/mmole.

[14α-Methyl-^{3}H]-24,25-dihydrolanosterol (6), electrochemical reduction

A solution of ketone (**19**) (81 mCi, 0.0009 mmole, 0.5 mg) in 35 mL dioxane was transferred to a lead cathode electrolysis cell. An equal volume of 10% sulfuric acid was added to the stirred solution of ketone. The anode compartment was charged with the same 1:1 solution of dioxane-10% sulfuric acid. Current was applied and increased from an initial 400 mA to 1200 mA over 12 hrs at which time no starting material remained as determined by TLC (20% ethyl acetate-hexane). The reaction solution and rinses of the cathode compartment were extracted three times with ether. The combined extracts were washed sequentially with $NaHCO_3$, water, and brine, then dried over Na_2SO_4. Purification by Chromatotron chromatography (1 mm silica gel rotor, 5-30% ethyl acetate-hexane gradient) afforded 50 mCi (67%) of pure product **6**. The specific activity was determined to be 8.3 Ci/mmole by mass spectroscopy. In addition, 13.7 mCi of the 15-alcohol, (**20**) was isolated.

The purity of **6** was found to be >99% by HPLC under the following

conditions: Vydac 201 HS 104 C-18, 4.5mm x 250mm, eluted with 50% i-PrOH-CH_3CN at 1 mL/min, monitored at 220nm, retention time 7.6 min.

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