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Biomimetic Synthesis of Petuniasterone D via the Epoxy Ester−Ortho Ester Rearrangement

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Received January 30, 2002

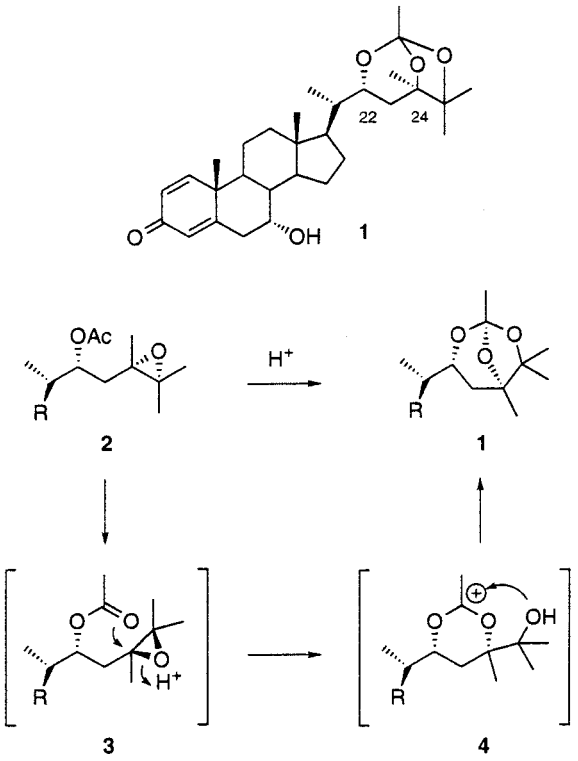
The side chain of the insecticidal steroid petuniasterone D was synthesized by the biomimetic acid-catalyzed epoxy ester−ortho ester rearrangement. In addition to the natural (22*R*,24*R*)-configuration of the side chain ortho ester, compounds bearing the epimeric (22*R*,24*S*)-, (22*S*,24*R*)-, and (22*S*, 24*S*)- [3.2.1]-bicyclic ortho esters were also produced by stereospecific rearrangement of the corresponding isomeric epoxy esters. Functionalization of the steroid nucleus of the (22*R*,24*R*)-ortho ester completed the synthesis of the natural product.

Introduction

Petuniasterone D (**1**) was one of the first members of a family of about 60 steroidal metabolites to be isolated from wild and commercial *Petunia* species in the laboratory of Carl Elliger.[1] The structures of these petuniasteroids were solved largely by X-ray crystallography, and 40 members of this group were shown to feature a [3.2.1]-bicyclic ortho ester system in the steroidal side chain.[2] These steroids are potent insecticides and exhibit ED_{50} values as low as 2 ppm against the larvae of various lepidopteran species.[3] Recently, the molluscicidal properties of petuniasteroids against freshwater snails have also been reported.[4] The insecticidal activity of these petuniasteroids requires the presence of the ortho ester and is believed to be mediated through the antagonism of the $GABA_a$ cyclodiene receptor.[5]

Due to the cooccurrence of epoxy esters and ortho esters within the plant, it was proposed that the biosynthesis of the ortho ester proceeds via the intermediacy of an epoxy ester.[2b] The conversion of petuniasterone C 22-acetate (**2**) into **1** was demonstrated in vitro in 60% yield using a solution of perchloric acid in dioxane.[2b] This rearrangement takes place by stereospecific 6-*exo* ring closure with inversion at the 24-position (**3**), followed by intramolecular quenching of the dioxycarbenium ion by the newly generated hydroxyl group (**4**, Scheme 1). Although similar reactions have long been known, relatively few examples have been reported.[6] We have recently made use of the epoxy ester-ortho ester rear-

SCHEME 1. Biogenetic Origin of Petuniasterone D Side Chain



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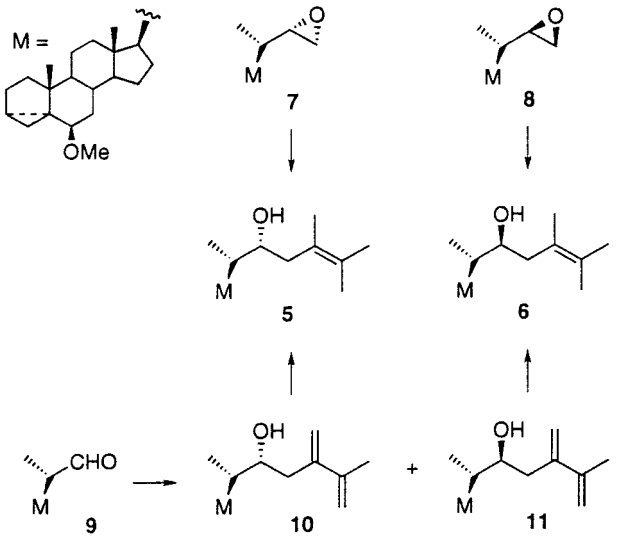
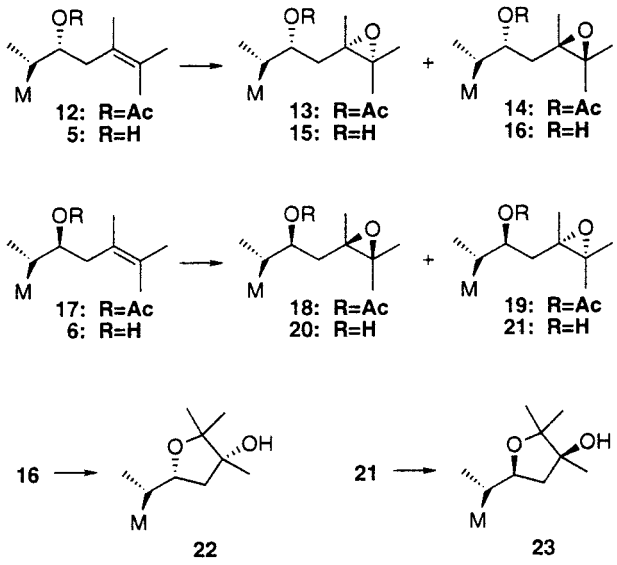
10.1021/jo020068s CCC: $22.00
Published on Web 05/17/2002

SCHEME 2



SCHEME 3



rangement in the biomimetic approach to ortho esterol B, an antiviral marine steroid from the sponge *Petrosia weinbergi*, and in the synthesis of 2-C-methyl-D-erythritol, an intermediate in the deoxyxylulose pathway of isoprenoid biosynthesis.[7]

In this paper we describe our development of a stereocontrolled, high yielding, and general route to the petuniasteroid side chain, in a manner mimicking the natural biosynthetic process. These efforts have culminated with the synthesis of petuniasterone D (**1**).

Results and Discussion

Our biomimetic construction of the bicyclic ortho ester moiety of petuniasterone D (**1**) began with the synthesis of homoallylic alcohols **5** and **6**. Initially attempts were made to couple the Grignard reagent derived from 2-bromo-3-methyl-2-butene or its lithium analogue with epoxides **7** and **8** (Scheme 2).[8] This approach, similar to that described for the synthesis of inotodiol,[9] led to the direct and stereospecific formation of each of the alcohols **5** and **6**. However, the reaction was poorly reproducible, and a more reliable preparation was achieved by treating steroidal aldehyde **9** with the allylpotassium generated from 2,3-dimethyl-1,3-butadiene.[10] Birch reduction of the resulting mixture of diene alcohols **10** and **11** gave alcohols **5** and **6** in a 1:2 mixture which was easily separable by flash chromatography in 81% overall yield. Attempts to gain more of the desired alcohol **5** by inversion of the epimeric 22-alcohol **6** using either the Corey or the Mitsunobu protocols were fruitless.[11]

Peracid epoxidation of the (22*R*)-acetate **12** obtained by acetylation of **5** led to a 1:4 mixture of epoxy acetates **13** and **14** (Scheme 3). The absolute stereochemistry of the minor epoxy acetate (**13**) could be established as the desired (22*R*,24*S*)-configuration by comparison of the ^{1}H NMR spectrum with the published data for petuniasterone C 22-acetate (**2**).[2a] Thus, the characteristic signals of the steroidal side chain of **13** (the C-22 proton at 5.23 ppm (dt, J = 11.0 and 2.0 Hz) and three methyls singlets at 1.36, 1.30, and 1.29 ppm) were in excellent agreement with those reported for **2**: 5.24 (dt, J = 11.0 and 2.0 Hz), 1.35 (s), 1.30 (s), and 1.28 (s).[2a] On the other hand, the NMR signals of the major (22*R*,24*R*)-epoxy acetate **14** (5.13 (dt, J = 11.4 and 3.0 Hz), 1.33 (s), 1.32 (s), and 1.28 (s) ppm) differed substantially from those of the natural (22*R*,24*S*)-epoxy ester **2**.

A reversal of stereoselectivity favoring the desired epoxide was observed when peracid epoxidation was carried out with the free alcohol **5**. This is due to the directing effect of the hydroxyl group which leads predominantly to *syn*-addition giving the epoxy alcohols **15** and **16** in a ca. 7:1 ratio.[12] An even greater improvement of stereocontrol was achieved when **5** was submitted to Sharpless's vanadium-catalyzed *tert*-butyl hydroperoxide epoxidation,[13] favoring the *syn*-isomer **15** in a >60:1 ratio with a 98% yield.

Similar results were obtained in the (22*S*)-series. Thus, application of peracid epoxidation of the (22*S*)-acetate **17** led to epoxy acetates **18** and **19** as a 1:2 mixture, while the (22*S*)-alcohol **6** provided epoxides **20** and **21** in a 4:1 ratio. In this case, Sharpless catalytic epoxidation was not as remarkable, leading to a 16:1 ratio favoring the *syn*-isomer **20**. In addition to the reactivity patterns, the

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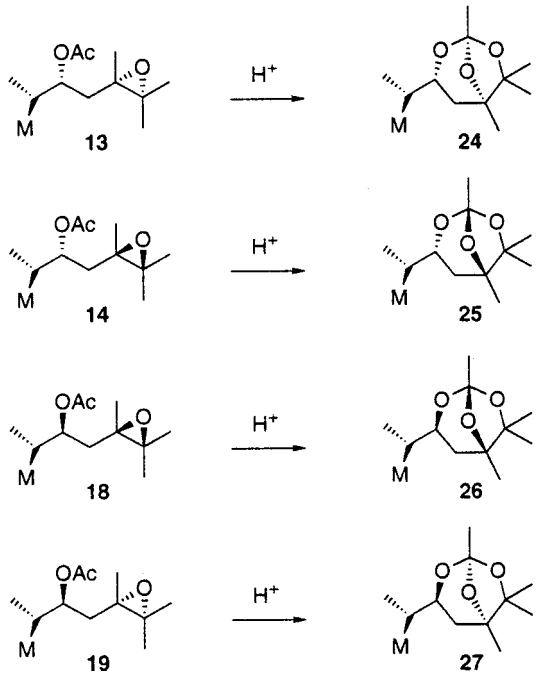
SCHEME 4



absolute stereochemistry of epoxy alcohols **20** and **21** could be assigned by correlation of their ^{1}H NMR spectra with their counterparts in the (22*R*)-series. Epoxy alcohol **20** displays chemical shifts related to its side chain at 4.14 (br d, J = 8.7 Hz), 1.37 (s), 1.36 (s), and 1.34 (s) ppm similar to the *syn*-isomer in the (22*R*)-series **15**: 4.17 (dt, J = 10, 3 Hz), 1.37 (6H, s), and 1.35 (s) ppm. A different pattern was observed for the *anti*-isomers, with NMR values of 3.94 (dd, J = 9.4 and 4.0 Hz), 1.37 (s) and 1.32 (6H, s) ppm for compound **21** and 3.88 (br d, J = 11.0), 1.38 (s), 1.34 (s), and 1.33 (s) ppm for **16**.

It was noted that the epoxy alcohols in the *anti* series (**16** and **21**) were not stable in $CDCl_3$ unless the solvent was purified by filtration through alumina, due to traces of acid. After 2 days in unpurified NMR solvent, these two epoxy alcohols had rearranged almost completely to tetrahydrofuranyl alcohols (**22** and **23**). The *syn*-epoxy alcohols were completely inert under the same conditions, although petuniasterone C, which has the side chain of **15**, has been shown to rearrange to the tetrahydrofuran petuniasterone F by treatment with perchloric acid.[2b]

Instability in $CDCl_3$ containing traces of HCl or DCl was also observed for the four epoxy acetates (**13**, **14**, **18**, and **19**) which rearranged to ortho esters. This reaction could also be carried out in purified $CDCl_3$ or benzene containing 0.05−0.2% TFA. Thus, compound **13** smoothly rearranged to an ortho ester that exhibited NMR signals for its side chain at 4.20 (dt, J = 11.3 and 4.4 Hz), 1.31 (s), 1.20 (s), 1.17 (s), and 0.97 (d, J = 6.6 Hz) ppm. These signals nicely matched the reported data for petuniasterone D (**1**) side chain at 4.20 (dt, J = 11.0 and 5.0 Hz), 1.30 (s), 1.20 (s) 1.16 (s), and 0.97 (d, J = 7.0 Hz) ppm, leading to the assignment of the (22*R*,24*R*)-configuration (**24**, Scheme 4).[1] Under the same mild acidic conditions, epoxy acetate **14** also rearranged cleanly with inversion at C-24, to a different ortho ester (**25**) having NMR signals at 3.84 (ddd, J = 9.6, 5.4, and 4.0 Hz), 1.33 (s), 1.27 (s), 1.15 (s), and 0.95 (d, J = 6.6 Hz) ppm, clearly different from those describing side chain of **1**.

The (22*S*)-epoxy acetates **18** and **19** also underwent stereospecific epoxy ester−ortho ester rearrangement leading to orthoacetates **26** and **27**, which show characteristic NMR signals at 4.19 (dd, J = 11.4 and 3.6 Hz), 1.31 (s), 1.19 (s), and 1.15 (s) ppm, and 3.83 (dd, J = 11.3 and 5.4 Hz), 1.32 (s), 1.29 (s) and 1.14 (s) ppm, respectively. We note here that the *anti*-epoxy acetates **14** and **19** were converted into ortho esters at a much higher rate than the *syn*-epoxy acetates **13** and **18**, and that within the either the *syn*- or *anti*-series, the (22*R*)-isomer reacted faster than the (22*S*)-isomer. The greater reactivity of the *anti*-isomers can be explained by the ability of both the bulky epoxy and steroidal groups to occupy equatorial positions in a chairlike transition state.

With a method in hand to generate the desired (22*R*, 24*R*)-bicyclic ortho ester side chain, our efforts focused on the manipulation of the steroid nucleus to complete the synthesis of **1**. Surprisingly, deprotection of the steroid nucleus could be accomplished quantitatively in the presence of the ortho ester moiety in **24** by using a 0.26 M solution of TFA in either $CDCl_3$ or benzene. Therefore, both ortho ester formation and deprotection of the nucleus could be carried out in one step from **13**. The resulting Δ^5 3β-trifluoroacetate intermediate was deprotected in situ (K_2CO_3/MeOH) to give Δ^5 3β-sterol **28** in 97% overall yield (Scheme 5). Access to the trienone system was obtained by treatment of **28** with DDQ in refluxing benzene to give **29** in 68% yield, accompanied by more polar byproducts that appeared to have lost the ortho ester.[14] By contrast, use of dioxane in the DDQ oxidation resulted in a 13% yield.

An alternative route to trienone **29** was developed to circumvent the difficulties associated with DDQ oxidation. Using this route, the ortho ester was generated last, and the oxidation of the nucleus was carried out in two steps to minimize the use of DDQ. Thus treatment of the homoallylic acetate **12** with 0.25 M TFA in benzene, followed by selective removal of the trifluoroacetate, gave the Δ^5 sterol **30** in 94% yield. Wettstein−Oppenauer oxidation gave the $\Delta^{4,6}$ 3-ketone **31** (92% yield),[15] which was subjected to DDQ treatment to give **32** (77% yield). To effect the selective epoxidation of side chain, acetate **32** was first saponified to the alcohol **33**. The reacetylated product of epoxidation (**34**) was then subjected to acid-catalyzed rearrangement to give ortho ester **29** (92% from acetate **32**). Although this detour involved more steps with about the same overall yield, the difficulties associated with the DDQ reaction in the presence of the ortho ester made this route preferable for larger scale reactions.

To complete the synthesis of the natural product, regio- and stereoselective epoxidation of the trienone **29** with *m*-CPBA afforded the 6α,7α-epoxide **35** in 99% yield.[16] Reductive epoxide ring-opening of **35** with aluminum amalgam,[16,17] followed by conjugation of the intermediate Δ^5 ketone with K_2CO_3/MeOH, gave petuniasterone D (**1**)

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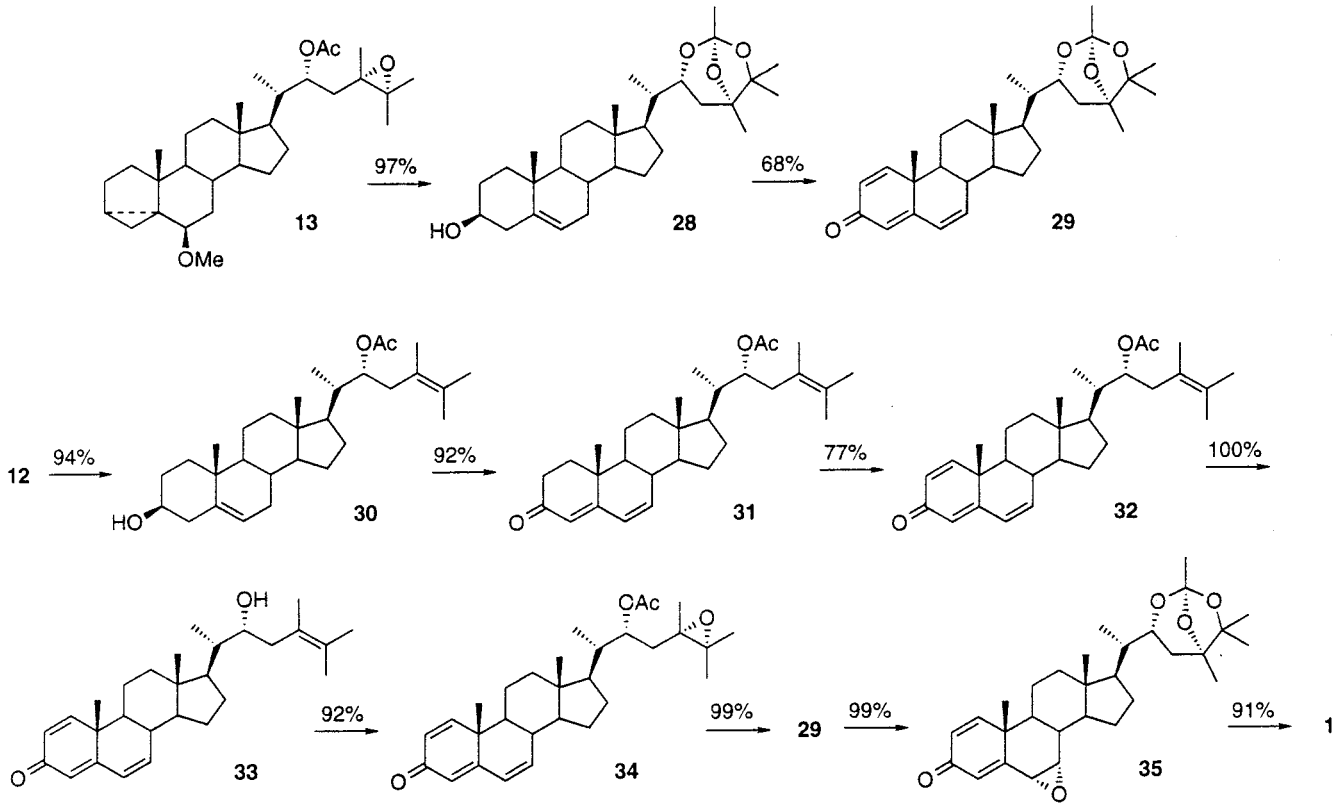
SCHEME 5



in 91% yield. Both ^{1}H and ^{13}C NMR spectra matched those of an authentic sample of petuniasterone D.

Conclusion

The biomimetic acid-catalyzed epoxy ester−ortho ester rearrangement provides a very efficient approach to the construction of bioactive bicyclic ortho esters. In the case of petuniasteroid ortho esters, this reaction was found to be completely stereospecific. This rearrangement has allowed the synthesis of the insecticidal natural product petuniasterone D (**1**).

By the fastest route, petuniasterone D was prepared in seven steps (**7** → **5** → **15** → **13** → **28** → **29** → **35** → **1**) with a 37% overall yield. However, the unreliability of the organometallic reagents derived from 2-bromo-3-methyl-2-butene led us to prefer the reaction of aldehyde **9** with an allylpotassium reagent, although the desired product **10** was the minor stereoisomeric product, and conditions could not be found to invert the hydroxyl of the epimeric **11**. Extra steps were also introduced to circumvent problems associated with the reactivity of the ortho ester in the presence of DDQ, leading again to a more reliable route. The improved route to petuniasterone D therefore requires 11 steps (**9** → **10** → **5** → **12** → **30** → **31** → **32** → **33** → **34** → **29** → **35** → **1**) and proceeds in a 15% overall yield, largely due to the 27% yield in the first step. This synthesis has permitted the preparation of several analogues of the natural product. Structure−activity studies are underway to correlate insecticidal activity with molecular parameters.

Experimental Section

General Methods. NMR spectra were acquired using 300 and 600 MHz instruments using $CDCl_3$ as the solvent. TLC was performed on aluminum-backed plates coated with a 0.25 mm layer of silica gel 60 F254. All reagents were obtained commercially and were used without further purification.

(22*R*)- and (22*S*)-6*β*-Methoxy-3α,5-cyclo-5α-ergost-24-en-22-ols (5 and 6): Via Grignard Reaction. To 120 mg of Mg metal (4.94 mmol) in 2 mL of THF were added 60 μL (0.57 mmol) 1,2-dibromopropane and 0.6 g (4.02 mmol) 2-bromo-3-methyl-2-butene. After 3 h at room temperature, the thick gray suspension was heated at 70 °C for another 2 h, and 1.5 mL was added to 137 mg (0.38 mmol) of a 1:4 mixture of the (22*R*)- (**7**) and (22*S*)- (**8**) isomers of 22,23-epoxy-6*β*-methoxy-3α,5-cyclo-5α-24-norcholane[18] dissolved in 1.5 mL of THF. After 24 h at room temperature, the reaction was quenched with 5% NaOH and extracted with 10% HCl and hexane. After evaporation of the solvent, the product was purified by flash chromatography (5−7.5% EtOAc−hexane) to give 22.8 mg of **5** (14% yield) and 82.0 mg of **6** (50% yield). A similar reaction with pure **8** led to pure **6**.

5: R_f 0.41 (20% EtOAc−hexane). $[\alpha]^{22}_D$ +40.5° (*c* 0.17, CH_2Cl_2). ^{1}H NMR (300 MHz): 3.76 (1H, br d, J = 11.1 Hz), 3.32 (3H, s), 2.77 (1H, s), 2.39 (1H, dd, J = 13.1, 11.4 Hz), 1.71 (6H, s), 1.66 (3H, s), 1.02 (3H, s), 0.98 (3H, d, J = 6.7 Hz), 0.75 (3H, s), 0.65 (1H, m), 0.44 (1H, m); ^{13}C NMR (75 MHz): 128.3 (s), 124.5 (s), 82.4 (d), 70.7 (d), 56.5 (q), 56.2 (d), 53.2 (d), 48.1 (d), 43.4 (s), 43.2 (s), 40.6 (d), 40.3 (t), 35.3 (s), 35.1 (t), 34.5 (t), 33.4 (t), 30.5 (d), 27.5 (t), 25.0 (t), 24.3 (t), 22.8 (t), 21.5 (d), 20.9 (q), 20.6 (q), 19.3 (q), 18.4 (q), 13.1 (t), 12.4 (q), 12.2 (q). MS (EI) *m*/*z* (relative intensity) 428 (0.2, M^+), 396 (0.3), 313 (12), 84 (100). Anal. Calcd for $C_{29}H_{48}O_2$: C 81.30; H 11.21. Found C 81.19; H 11.33.

6: R_f 0.45 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 3.79 (1H, dd, J = 8.2, 3.6 Hz), 3.32 (3H, s), 2.76 (1H, s), 2.47 (1H, dd, J = 13.4, 9.2 Hz), 1.68 (6H, s), 1.67 (3H, s), 1.02 (3H, s), 0.96 (3H, d, J = 6.5 Hz), 0.71 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 127.4 (s), 124.8 (s), 82.4 (d), 71.6 (d), 56.6 (q), 56.4 (d), 53.1 (d), 48.0 (d), 43.4 (s), 42.7 (s), 40.4 (d),

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40.3 (t), 40.2 (t), 35.3 (s), 35.1 (t), 33.4 (t), 30.6 (d), 27.9 (t), 25.0 (t), 24.2 (t), 22.8 (t), 21.5 (d), 20.8 (q), 20.6 (q), 19.3 (q), 18.8 (q), 13.1 (t), 12.1 (q), 12.1 (q). Anal. Calcd for $C_{29}H_{48}O_2$: C 81.30; H 11.21. Found C 80.98; H 11.37.

Via Organolithium Reaction. A solution of 0.8 mL (6.89 mmol) 2-bromo-3-methyl-2-butene in 1.5 mL of dry THF was added to 79 mg of Li wire (11.45 mmol) in 3 mL of dry THF at −10 °C and under Ar. The temperature slowly increased to 0 °C over 45 min and then was held at 0 °C for another 20 min. A solution of 630 mg (1.76 mmol) epoxide **7** in 3 mL of THF was added dropwise. After 48 h at room temperature, the reaction was quenched with H_2O and extracted with Et_2O. After evaporation of the solvent, the product was purified by silica gel chromatography to give 260 mg of **7** (41% recovery), and 189 mg of **5** (25% yield).

Via Allylpotassium Reaction. To a mixture of 1.2 g (10.7 mmol) of KO*t*-Bu and 1.6 mL (9.5 mmol) of 2,2,6,6-tetramethylpiperidine in 20 mL of THF was added 6.4 mL (10.24 mmol) of 1.6 M n-BuLi in hexane at −78 °C under N_2. The resulting orange solution was stirred for 45 min until all KO*t*-Bu was dissolved, and 2 mL of (17.7 mmol) of 2,3-dimethyl-1,3-butadiene was added, resulting in a deep red solution. After another 45 min, a solution of 0.92 g (2.67 mmol) of (20*R*)-6*β*-methoxy-3α,5-cyclo-5α-pregnane 20-carboxaldehyde (**9**) was added in 4 mL of THF. After 30 min, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4, and the solvents were removed under reduced pressure to give a 1:2 mixture of dienes **10** and **11** (1.23 g) as a brown oil. A small portion of was purified by preparative TLC for NMR characterization. A solution of the crude diene alcohols **10** and **11** (1.20 g) in 10 mL of THF was added dropwise to a 0.35 g of Li (50.7 mmol) in 60 mL of liq NH_3. After 1 h, the reaction was quenched with 20 mL of EtOH/Et_2O 1:1. The NH_3 was allowed to evaporate, and the mixture was extracted with H_2O and EtOAc. The organic layer was dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. Purification by flash chromatography (5% EtOAc−hexane) gave 303 mg of **5** (27% yield) and 620 mg of **6** (54% yield).

(22*R*)-6*β*-Methoxy-3α,5-cyclo-5α-ergosta-24(28),25-dien-22-ol (10): R_f 0.40 (20% AcOEt−hexane); ^{1}H NMR (300 MHz): 5.24 (1H, s), 5.10 (1H, s), 5.06 (1H, s), 5.04 (1H, s), 3.75 (1H, br d, J = 10.7 Hz), 3.33 (3H, s), 2.77 (1H, br s), 2.53 (1H, d, J = 13.8 Hz), 1.94 (3H, s), 1.02 (3H, s), 0.98 (3H, d, J = 6.8 Hz), 0.76 (3H, s), 0.65 (1H, t, J = 4.8 Hz), 0.43 (1H, dd, J = 8.0, 4.1 Hz).

(22*S*)-6*β*-Methoxy-3α,5-cyclo-5α-ergosta-24(28),25-dien-22-ol (11): R_f 0.48 (20% AcOEt−hexane); ^{1}H NMR (300 MHz): 5.19 (1H, s), 5.05 (1H, s), 5.04 (1H, s), 5.02 (1H, s), 3.78 (1H, t, J = 6.5 Hz), 3.33 (3H, s), 2.77 (1H, t, J = 2.8 Hz), 2.40 (2H, d, J = 6.3 Hz), 1.92 (3H, s), 1.02 (3H, s), 0.96 (3H, d, J = 6.3 Hz), 0.72 (3H, s), 0.65 (1H, t, J = 4.9 Hz), 0.43 (1H, dd, J = 8.1, 4.5 Hz).

(22*R*)-6*β*-Methoxy-3α,5-cyclo-5α-ergost-24-en-22-yl Acetate (12). By acetylation of **5** (see **13**). R_f 0.52 (10% EtOAc−hexane). $[\alpha]^{22}_D$ +33.9° (*c* 0.16, CH_2Cl_2). ^{1}H NMR (300 MHz): 5.05 (1H, d, J = 11.4 Hz), 3.32 (3H, s), 2.76 (1H, s), 2.43 (1H, dd, J = 13.8, 11.4 Hz), 1.95 (3H, s), 1.65 (3H, s), 1.61 (6H, s), 1.01 (3H, s), 0.98 (3H, d, J = 6.7 Hz), 0.72 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 170.4 (s), 126.6 (s), 124.1 (s), 82.4 (d), 75.1 (d), 56.6 (q), 56.2 (d), 53.2 (d), 48.0 (d), 43.4 (s), 43.2 (s), 40.3 (t), 39.7 (d), 35.3 (s), 35.0 (t), 33.4 (t), 32.1 (t), 30.5 (d), 27.4 (t), 25.0 (t), 24.3 (t), 22.8 (t), 21.5 (d), 21.3 (q), 20.8 (q), 20.5 (q), 19.3 (q), 18.9 (q), 13.1 (t), 13.1 (q), 12.2 (q). Anal. Calcd for $C_{31}H_{50}O_3$: C 79.14; H 10.63. Found C 78.82; H 10.73.

(22*R*,24*S*)-24,25-Epoxy-6*β*-methoxy-3α,5-cycloergostan-22-yl Acetate (13). Peracid epoxidation of **12** (see **15**) gave **13** and **14** in a 1:4 ratio. Alternatively, acetylation of 203 mg of epoxide **15** with a 3:1 mixture of pyridine−acetic anhydride (2 mL) at room temperature for 24 h gave, after evaporation under reduced pressure, 221 mg of **13** (99% yield). R_f 0.44 (20% EtOAc−hexane). $[\alpha]^{22}_D$ +44.5° (*c* 0.37, CH_2Cl_2). ^{1}H NMR (300 MHz) 5.23 (1H, dt, J = 11, 2 Hz), 3.32 (3H, s), 2.77 (1H, t, J = 2.7 Hz), 2.06 (3H, s), 1.36 (3H, s), 1.30 (3H, s), 1.29 (3H, s), 1.02 (3H, s), 0.94 (3H, d, J = 6.7 Hz), 0.72 (3H, s), 0.65 (1H, m), 0.43 (1H, m). ^{13}C NMR (75 MHz): 170.6 (s), 82.3 (d), 73.4 (d), 63.0 (s), 61.4 (s), 56.6 (q), 56.3 (d), 52.9 (d), 48.0 (d), 43.4 (s), 43.2 (s), 40.2 (t), 40.2 (d), 35.3 (s), 35.1 (t), 33.4 (t), 32.8 (t), 30.5 (d), 27.5 (t), 25.0 (t), 24.3 (t), 22.7 (t), 21.6 (q), 21.5 (d), 21.3 (q), 21.2 (q), 19.6 (q), 19.3 (q), 13.1 (t), 12.9 (q), 12.2 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.27; H 10.24.

(22*R*,24*R*)-24,25-Epoxy-6*β*-Methoxy-3α,5-cyclo-5α-ergostan-22-yl Acetate (14): R_f 0.52 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 5.13 (1H, dt, J = 11.4, 3.0 Hz), 3.32 (3H, s), 2.77 (1H, s), 2.05 (3H, s), 1.33 (3H, s), 1.32 (3H, s), 1.28 (3H, s), 1.02 (3H, s), 0.92 (3H, d, J = 6.7 Hz), 0.72 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 170.2 (s), 82.4 (d), 73.6 (d), 63.2 (s), 62.8 (s), 56.6 (q), 56.1 (d), 53.1 (d), 48.0 (d), 43.4 (s), 43.1 (s), 40.2 (t), 39.3 (d), 35.3 (s), 35.0 (t), 33.4 (t), 32.7 (t), 30.5 (d), 27.1 (t), 25.0 (t), 24.2 (t), 22.7 (t), 21.4 (q), 21.5 (d), 21.3 (q), 21.3 (q), 19.3 (q), 19.1 (q), 13.1 (t), 12.9 (q), 12.3 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.18; H 10.22.

(22*R*,24*S*)- and (22*R*,24*R*)-24,25-Epoxy-6*β*-methoxy-3α,5-cycloergostan-22-ol (15 and 16). A stirred solution of 47 mg of alcohol **5** (0.11 mmol) in 2 mL of CH_2Cl_2 was treated with 57 mg (0.24 mmol) of *m*-CPBA (75%) at room temperature for 2 min. The reaction was quenched with 2 mL of 5% NaOH and extracted with Et_2O. The organic layer was dried over Na_2SO_4, and the solvents were removed under reduced pressure. Purification by flash chromatography (hexanes−EtOAc 29:1) gave 40 mg of **15** (82% yield) and 5.6 mg of **16** (11% yield).

Alternatively, to 16.8 mg (0.04 mmol) of **5** in 0.5 mL of dry benzene at room temperature was added 20 μL (1.4 μmol) of 70 mM vanadyl acetylacetonate in benzene, followed after 5 min by 10 μL (0.05−0.06 mmol) of 5−6 M *tert*-butyl hydroperoxide in decane. After 1 h, 1 mL of 5% NaOH was added, and the mixture extracted with hexanes−EtOAc 2:1. After filtration through silica gel and evaporation, 17.1 mg of a >60:1 mixture of **15** and **16** was obtained (98% yield).

15: R_f 0.44 (20% EtOAc−hexane). $[\alpha]^{22}_D$ +41.9° (*c* 0.48, CH_2Cl_2). ^{1}H NMR (300 MHz) 4.17 (1H, dt, J = 10, 3 Hz), 3.32 (3H, s), 2.77 (1H, br s), 1.37 (6H, s), 1.35 (3H, s), 1.03 (3H, s), 0.92 (3H, d, J = 6.7 Hz), 0.76 (3H, s), 0.65 (1H, m), 0.43 (1H, m). ^{13}C NMR (75 MHz): 82.3 (d), 71.2 (d), 65.4 (s), 62.4 (s), 56.5 (q), 56.2 (d), 53.2 (d), 48.1 (d), 43.4 (s), 43.2 (s), 40.9 (d), 40.3 (t), 35.3 (s), 35.1 (t), 33.4 (t), 31.5 (t), 30.5 (d), 27.6 (t), 25.0 (t), 24.4 (t), 22.8 (t), 21.5 (d), 21.5 (q), 20.8 (q), 19.5 (q), 19.3 (q), 13.1 (t), 12.5 (q), 12.3 (q). MS (EI) *m*/*z* (relative intensity) 444 (5, M^+), 429 (13) 412 (6), 389 (20), 284 (55), 129 (100), 111 (64), 85 (84). Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.13; H 10.99.

16: R_f 0.30 (20% EtOAc−hexane); ^{1}H NMR (300 MHz) 3.88 (1H, br d, J = 11.0 Hz), 3.32 (3H, s), 2.77 (1H, t, J = 2.7 Hz), 1.38 (3H, s), 1.34 (3H, s), 1.33 (3H, s), 1.02 (3H, s), 0.93 (3H, d, J = 6.7 Hz), 0.74 (3H, m), 0.65 (1H, m), 0.43 (1H, m). ^{13}C NMR (75 MHz): 82.4 (d), 70.7 (d), 63.7 (s), 63.1 (s), 56.6 (q), 56.1 (d), 53.3 (d), 48.0 (d), 43.4 (s), 43.1 (s), 42.4 (d), 40.3 (t), 35.3 (s), 35.0 (t), 34.1 (t), 33.4 (t), 30.5 (d), 27.5 (t), 25.0 (t), 24.3 (t), 22.7 (t), 21.7 (q), 21.5 (d), 21.3 (q), 19.9 (q), 19.3 (q), 13.1 (t), 12.2 (q), 12.2 (q).

(22*S*)-6*β*-Methoxy-3α,5-cyclo-5α-ergost-24-en-22-yl Acetate (17): By acetylation of **6** (see **13**). R_f 0.65 (10% EtOAc−hexane); ^{1}H NMR (300 MHz): 5.12 (1H, t, J = 7.0 Hz), 3.32 (3H, s), 2.76 (1H, s), 2.44 (1H, dd, J = 13.4, 8.1 Hz), 1.98 (3H, s), 1.66 (6H, s), 1.62 (3H, s), 1.01 (3H, s), 1.01 (3H, d, J = 6.6 Hz), 0.70 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 170.6 (s), 127.0 (s), 123.8 (s), 82.4 (d), 74.8 (d), 56.6 (q), 56.4 (d), 53.0 (d), 48.0 (d), 43.4 (s), 42.7 (s), 40.2 (t), 39.0 (d), 37.1 (t), 35.3 (s), 34.9 (t), 33.4 (t), 30.5 (d), 28.3 (t), 25.0 (t), 24.1 (t), 22.8 (t), 21.5 (d), 21.1 (q), 20.7 (q), 20.5 (q), 19.3 (q),

18.9 (q), 13.2 (q), 13.0 (t), 12.0 (q). Anal. Calcd for $C_{31}H_{50}O_3$: C 79.14; H 10.63. Found C 78.96; H 10.68.

(22*S*,24*R*)-24,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-22-yl Acetate (18). Peracid epoxidation of **17** (see **15**) gave **18** and **19** in a 1:2 ratio. Alternatively, acetylation of **20** and **21** (see **13**) gave **18** and **19**, respectively. R_f 0.61 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 5.23 (1H, dd, J = 8.2, 4.9 Hz), 3.32 (3H, s), 2.76 (1H, s), 2.05 (3H, s), 1.34 (3H, s), 1.31 (3H, s), 1.29 (3H, s), 1.01 (3H, s), 0.98 (3H, d, J = 6.7 Hz), 0.71 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 170.7 (s), 82.4 (d), 73.5 (d), 62.7 (s), 61.9 (s), 56.6 (q), 56.4 (d), 52.7 (d), 48.0 (d), 43.3 (s), 42.7 (s), 40.4 (d), 40.2 (t), 37.7 (t), 35.3 (s), 35.0 (t), 33.4 (t), 30.5 (d), 28.3 (t), 24.9 (t), 24.1 (t), 22.8 (t), 21.5 (q), 21.5 (d), 21.3 (q), 21.1 (q), 20.2 (q), 19.3 (q), 13.2 (q), 13.1 (t), 12.0 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.33; H 10.58.

(22*S*,24*S*)-24,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-22-yl Acetate (19). ^{1}H NMR (300 MHz): R_f 0.54 (20% EtOAc−hexane); 5.23 (1H, dd, J = 9.0, 4.2 Hz), 3.32 (3H, s), 2.77 (1H, s), 2.05 (3H, s), 1.38 (3H, s), 1.29 (3H, s), 1.27 (3H, s), 1.02 (3H, s), 0.97 (3H, d, J = 6.7 Hz), 0.73 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 170.5 (s), 82.4 (d), 73.7 (d), 63.0 (s), 62.2 (s), 56.6 (q), 56.4 (d), 52.8 (d), 48.0 (d), 43.4 (s), 42.7 (s), 40.4 (d), 40.3 (t), 37.7 (t), 35.3 (s), 35.0 (t), 33.4 (t), 30.5 (d), 28.2 (t), 25.0 (t), 24.2 (t), 22.8 (t), 21.5 (d), 21.3 (q), 21.3 (q), 21.2 (q), 19.3 (q), 19.0 (q), 13.2 (q), 13.1 (t), 12.0 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.55; H 10.37.

(22*S*,24*R*)-24,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-22-ol (20). Peracid epoxidation of **6** (see **15**) gave **20** and **21** in a 4:1 ratio.

Alternatively, vanadium-catalyzed epoxidation of **6** (see **15**) gave **20** and **21** in a 16:1 ratio.

R_f 0.43 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 4.14 (1H, d, J = 8.7 Hz), 3.32 (3H, s), 2.77 (1H, s), 1.37 (3H, s), 1.36 (3H, s), 1.34 (3H, s), 1.02 (3H, s), 0.92 (3H, d, J = 6.6 Hz), 0.72 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 82.5 (d), 71.1 (d), 65.1 (s), 62.4 (s), 56.6 (q), 56.3 (d), 52.5 (d), 47.9 (d), 43.4 (s), 42.6 (s), 42.0 (d), 40.2 (t), 37.8 (t), 35.3 (s), 35.1 (t), 33.3 (t), 30.6 (d), 27.8 (t), 24.9 (t), 24.1 (t), 22.8 (t), 21.5 (d), 21.5 (q), 20.9 (q), 19.8 (q), 19.3 (q), 13.1 (t), 12.3 (q), 12.1 (q).

(22*S*,24*S*)-24,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-22-ol (21): R_f 0.33 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 3.94 (1H, dd, J = 8.4, 4.0 Hz), 3.32 (3H, s), 2.77 (1H, s), 1.37 (3H, s), 1.32 (6H, s), 1.02 (3H, s), 0.91 (3H, d, J = 6.1 Hz), 0.73 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 82.4 (d), 70.9 (d), 63.4 (s), 62.5 (s), 56.6 (q), 56.4 (d), 52.8 (d), 48.0 (d), 43.4 (s), 42.7 (s), 41.0 (d), 40.3 (t), 39.5 (t), 35.2 (s), 35.1 (t), 33.4 (t), 30.6 (d), 27.9 (t), 24.9 (t), 24.1 (t), 22.8 (t), 21.5 (q), 21.5 (d), 21.2 (q), 19.8 (q), 19.3 (q), 13.1 (t), 12.2 (q), 11.9 (q).

(22*R*,24*R*)-22,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-24-ol (22). The epoxy alcohol **16** isomerized cleanly to the tetrahydrofuran **22** when treated with unpurified $CDCl_3$ (containing traces of DCl). Evaporation of the solvent gave the product. R_f 0.41 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 4.06 (1H, ddd, J = 8.7, 6.8, 4.2 Hz), 3.32 (3H, s), 2.77 (1H, s), 1.24 (3H, s), 1.21 (3H, s), 1.14 (3H, s), 1.02 (3H, s), 0.97 (3H, d, J = 6.6 Hz), 0.73 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 84.1 (s), 82.4 (d), 80.2 (s), 75.6 (d), 56.5 (q), 56.1 (d), 53.7 (d), 48.0 (d), 43.4 (s), 43.2 (s), 40.2 (t), 40.0 (t), 37.7 (d), 35.3 (s), 35.0 (t), 33.4 (t), 30.5 (d), 27.6 (t), 25.0 (t), 24.2 (t), 22.9 (q), 21.7 (t), 21.7 (q), 21.5 (d), 21.4 (q), 19.3 (q), 13.1 (t), 12.5 (q), 12.3 (q).

(22*S*,24*S*)-22,25-Epoxy-6*β*-methoxy-3α,5-cyclo-5α-ergostan-24-ol (23). The tetrahydrofuran was produced cleanly by isomerization of epoxy alcohol **21** in acidic $CDCl_3$ (see **22**). R_f 0.55 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 4.06 (1H, t, J = 7.7 Hz), 3.32 (3H, s), 2.77 (1H, s), 1.22 (3H, s), 1.19 (3H, s), 1.12 (3H, s), 1.02 (3H, s), 1.00 (3H, d, J = 6.7 Hz), 0.71 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 84.2 (s), 82.4 (d), 80.3 (s), 75.6 (d), 56.5 (q), 56.2 (d), 53.1 (d), 48.0 (d), 44.0 (t), 43.4 (s), 42.8 (s), 40.1 (t), 39.3 (d), 35.3 (s), 35.1 (t), 33.3 (t), 30.6 (d), 28.0 (t), 25.0 (t), 24.1 (t), 22.8 (t), 22.6 (q), 21.5 (d), 21.3 (q), 21.3 (q), 19.3 (q), 13.0 (t), 12.5 (q), 12.1 (q).

(22*R*,24*R*)-6*β*-Methoxy-3α,5-cyclo-5α-ergostane 22,24,-25-Orthoacetate (24). By acidic $CDCl_3$ isomerization of **13** (see **22**). Alternatively, **13** was treated with 20 mL of 0.05% TFA in benzene (v/v) for 3 h at room temperature Evaporation of the solvent under reduced pressure (30 °C), followed by filtration through silica gel in 5% EtOAc−hexane, gave 365 mg of **24** (92% yield). R_f 0.62 (20% EtOAc−hexane); ^{1}H NMR (300 MHz): 4.20 (1H, dt, J = 11.3, 4.4 Hz), 3.32 (3H, s), 2.76 (1H, s), 1.56 (3H, s), 1.31 (3H, s), 1.20 (3H, s), 1.17 (3H, s), 1.02 (3H, s), 0.97 (3H, d, J = 6.6 Hz), 0.73 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 117.4 (s), 82.4 (s), 82.3 (d), 81.3 (s), 70.0 (d), 56.5 (q), 56.2 (d), 52.5 (d), 48.1 (d), 43.4 (s), 43.2 (s), 40.2 (t), 38.5 (d), 35.3 (s), 35.1 (t), 33.3 (t), 30.5 (d), 30.3 (t), 27.4 (t), 25.2 (q), 25.0 (t), 24.3 (t), 23.6 (q), 22.7 (t), 21.5 (d), 20.5 (q), 20.0 (q), 19.3 (q), 13.1 (t), 12.7 (q), 12.2 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.42; H 10.18.

(22*R*,24*S*)-6*β*-Methoxy-3α,5-cyclo-5α-ergostane 22,24,-25-Orthoacetate (25). By isomerization of **14** (see **24**). ^{1}H NMR (300 MHz): 3.84 (1H, ddd, J = 9.6, 5.4, 4.0 Hz), 3.32 (3H, s), 2.76 (1H, s), 2.19 (1H, dd, J = 13.8, 11.2 Hz), 1.56 (3H, s), 1.33 (3H, s), 1.27 (3H, s), 1.15 (3H, s), 1.02 (3H, s), 0.95 (3H, d, J = 6.6 Hz), 0.73 (3H, s), 0.65 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 117.1 (s), 82.4 (d), 81.0 (s), 80.9 (s), 67.7 (d), 56.5 (q), 56.2 (d), 52.4 (d), 48.1 (d), 43.4 (s), 43.2 (s), 40.2 (t), 38.4 (d), 35.3 (s), 35.0 (t), 33.3 (t), 31.4 (t), 30.5 (d), 27.4 (t), 25.0 (t), 24.8 (q), 24.3 (t), 24.2 (q), 22.8 (q), 22.7 (t), 21.9 (q), 21.5 (d), 19.3 (q), 13.1 (q), 13.0 (t), 12.2 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.24; H 10.46.

(22*S*,24*S*)-6*β*-Methoxy-3α,5-cyclo-5α-ergostane 22,24,-25-Orthoacetate (26). By isomerization of **18** (see **24**). ^{1}H NMR (300 MHz): 4.19 (1H, dd, J = 11.4, 3.6 Hz), 3.32 (3H, s), 2.77 (1H, s), 1.52 (3H, s), 1.31 (3H, s), 1.19 (3H, s), 1.15 (3H, s), 1.02 (3H, s), 0.95 (3H, d, J = 6.7 Hz), 0.70 (3H, s), 0.64 (1H, m), 0.43 (1H, m); ^{13}C NMR (75 MHz): 117.3 (s), 82.4 (d), 82.2 (s), 81.4 (s), 69.9 (d), 56.6 (q), 56.3 (d), 52.3 (d), 48.0 (d), 43.4 (s), 42.6 (s), 40.1 (t), 39.5 (d), 35.3 (s), 35.0 (t), 34.7 (t), 33.4 (t), 30.6 (d), 27.9 (t), 25.2 (q), 25.0 (t), 24.1 (t), 23.6 (q), 22.8 (t), 21.5 (d), 20.5 (q), 19.9 (q), 19.3 (q), 13.1 (t), 12.9 (q), 12.1 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.08; H 10.19.

(22*S*,24*R*)-6*β*-Methoxy-3α,5-cyclo-5α-ergostane 22,24,-25-Orthoacetate (27). By isomerization of **19** (see **24**). ^{1}H NMR (300 MHz): 3.83 (1H, dd, J = 11.3, 5.4 Hz), 3.32 (3H, s), 2.77 (1H, s), 2.32 (1H, dd, J = 13.3, 11.4 Hz), 1.52 (3H, s), 1.32 (3H, s), 1.29 (3H, s), 1.14 (3H, s), 1.02 (3H, s), 0.93 (3H, d, J = 6.7 Hz), 0.70 (3H, s), 0.64 (1H, m), 0.42 (1H, m); ^{13}C NMR (75 MHz): 117.1 (s), 82.5 (d), 81.1 (s), 80.8 (s), 67.5 (d), 56.5 (q), 56.2 (d), 52.6 (d), 48.0 (d), 43.4 (s), 42.6 (s), 40.1 (t), 38.5 (d), 36.0 (t), 35.3 (s), 35.0 (t), 33.4 (t), 30.6 (d), 27.7 (t), 25.0 (t), 25.0 (t), 24.8 (q), 24.1 (q), 23.0 (q), 22.8 (t), 21.5 (d), 21.5 (q), 19.3 (q), 13.3 (q), 13.0 (t), 12.1 (q). Anal. Calcd for $C_{31}H_{50}O_4$: C 76.54; H 10.28. Found C 76.23; H 10.34.

(22*R*,24*R*)-Ergost-5-en-3*β*-ol 22,24,25-Orthoacetate (28). Epoxy acetate **13** (1.7 g, 3.50 mmol) in 125 mL of dry benzene was treated with 125 mL of 2% TFA in benzene. After 20 min at room temperature, the solvents were removed under reduced pressure (30 °C), and 200 mL of MeOH and 2.5 g (18 mmol) of K_2CO_3 were added. After stirring for 50 min, the solids were filtered, the solvent was removed under reduced pressure, and the product was purified by silica gel chromatography (5−20% EtOAc−hexane) to give 1.61 g of **28** (97% yield). Similar treatment of **24** gave the same product.

28: R_f 0.54 (33% EtOAc−hexane). $[\alpha]^{22}_D$ +4.6° (c 0.09, CH_2Cl_2). ^{1}H NMR (300 MHz) 5.33 (1H, m), 4.18 (1H, dt, J = 11.2, 4.3 Hz), 3.50 (1H, m), 1.54 (3H, s), 1.29 (3H, s), 1.18 (3H, s), 1.16 (3H, s), 1.00 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 0.69 (3H, s). ^{13}C NMR (75.5 MHz) 140.8 (s), 121.3 (d), 117 2 (s), 82.3 (s),

81.2 (s), 71.6 (d), 69.8 (d), 56.3 (d), 52.2 (d), 50.1 (d), 42.6 (s), 42.2 (t), 39.6 (t), 38.5 (d), 37.2 (t), 36.4 (s), 31.8 (d), 31.8 (t), 31.5 (t), 30.2 (t), 27.2 (t), 25.1 (q), 24.3 (t), 23.4 (q), 20.9 (t), 20.4 (q), 19.9 (q), 19.2 (q), 12.6 (q), 11.7 (q). MS (EI) *m*/*z* (relative intensity) 412 (2, $M^+ - 60$), 171 (20), 111 (100). Anal. Calcd for $C_{30}H_{48}O_4$: C 76.27; H 10.17. Found C 76.10; H 10.07.

(22*R*,24*R*)-Ergosta-1,4,6-trien-3-one 22,24,25-Orthoacetate (29). Orthoacetate **28** (10.1 mg, 0.02 mmol) was treated with 169 mg (0.74 mmol) of DDQ in 1.5 mL of benzene at reflux under N_2. After 14 h, the reaction was worked up by adding 4 mL of 5% NaOH and extracting with Et_2O. The organic layer was dried over Na_2SO_4, and the solvents were removed with a stream of N_2. Purification by flash chromatography (CH_2Cl_2; hexanes−EtOAc 4:1) gave 6.4 mg of **29** (68% yield). See alternative preparation from **34** below.

29: R_f 0.46 (33% EtOAc−hexane). $[\alpha]^{22}_D$ +9.6° (*c* 0.10, CH_2Cl_2). ^{1}H NMR (300 MHz) 7.06 (1H, d, *J* = 10.1 Hz), 6.23 (2H, m), 6.02 (1H, d, *J* = 11.8 Hz), 6.00 (1H, s), 4.19 (1H, dt, *J* = 11.3, 4.3 Hz), 1.56 (3H, s), 1.30 (3H, s), 1.19 (6H, s), 1.17 (3H, s), 0.97 (3H, d, *J* = 6.6 Hz), 0.80 (3H, s). ^{13}C NMR (75.5 MHz) 186.2 (s), 162.5 (s), 152.8 (d), 138.2 (d), 128.0 (d), 127.5 (d), 123.7 (d), 117.2 (s), 82.3 (s), 81.2 (s), 69.7 (d), 53.2 (d), 52.0 (d), 48.3 (d), 43.3 (s), 41.1 (s), 39.3 (t), 38.5 (d), 38.1 (d) 30.2 (t), 27.1 (t), 25.1 (q), 23.7 (t), 23.4 (q), 21.7 (t), 20.6 (q), 20.4 (q), 19.9 (q), 12.6 (q), 11.8 (q). MS (EI) *m*/*z* (relative intensity) 466 (4, M^+), 406 (4), 324 (5), 171 (37), 111 (100). Anal. Calcd for $C_{30}H_{42}O_4$: C 78.90; H 10.59. Found C 79.00; H 10.59.

(22*R*)-22-Acetoxyergosta-5,24-dien-3*β*-ol (30). Steroid **12** (6.07 g, 12.78 mmol) was treated with 80 mL of 0.25 M TFA in benzene for 15 min at room temperature The solvent was removed under reduced pressure, and the residue was treated with 4.5 g (33 mmol) of K_2CO_3 in 250 mL of MeOH at room temperature. After stirring for 5 min, the solids were removed by filtration, and the solution was extracted with H_2O and Et_2O. The organic layer was dried with Na_2SO_4, and the solvents were removed under reduced pressure to give 5.5 g of **30** (94% yield). R_f 0.48 (33% EtOAc−hexane). $[\alpha]^{22}_D$ −36.9° (*c* 0.64, CH_2Cl_2). ^{1}H NMR (600 MHz) 5.35 (1H, m), 5.06 (1H, dt, *J* = 11.5, 2.6 Hz), 3.52 (1H, m), 2.44 (1H, dd, *J* = 13.9, 11.4 Hz), 2.31−2.23 (2H, m), 1.95 (3H, s), 1.66 (3H, s), 1.63 (3H, s), 1.62 (3H, s), 1.01 (3H, s), 1.00 (3H, d, *J* = 6.8 Hz), 0.69 (3H, s). ^{13}C NMR (151 MHz) 170.3 (s), 140.7 (s), 126.6 (s), 124.0 (s), 121.6 (d), 75.1 (d), 71.7 (d), 56.4 (d), 53.0 (d), 50.1 (d), 42.7 (s), 42.3 (t), 39.8 (t), 39.6 (d), 37.2 (t), 36.5 (s), 32.1 (t), 31.9 (d), 31.8 (t), 31.6 (t), 27.2 (t), 24.4 (t), 21.3 (q), 21.1 (t), 20.7 (q), 20.4 (q), 19.3 (q), 18.8 (q), 13.1 (q), 11.8 (q). Anal. Calcd for $C_{30}H_{48}O_3$: C 77.25; H 9.01. Found C 77.37; H 9.04.

(22*R*)-22-Acetoxyergosta-4,6,24-trien-3-one (31). To 50 mL of benzene were added 401 mg (0.88 mmol) of **30** and 1.0 g (9.26 mmol) of *p*-benzoquinone. To ensure anhydrous conditions, 20 mL of benzene was distilled off, and then 0.5 g (2.5 mmol) of Al(*i*-PrO)$_3$ was added. The resulting purple solution was refluxed under N_2 for 5 h. The solids were filtered off, and the solvent was evaporated under reduced pressure. The residue was dissolved in 100 mL of Et_2O and extracted with two 400 mL portions of 5% NaOH. The organic layer was washed with brine, 10% HCl, and brine. After being dried with Na_2SO_4, the solvents were evaporated under reduced pressure, and the product purified by flash chromatography (5% EtOAc−hexane) to give 364 mg of **31** (92% yield). R_f 0.59 (33% EtOAc−hexane). $[\alpha]^{22}_D$ +15.7° (*c* 0.12, CH_2Cl_2). ^{1}H NMR (600 MHz) 6.14−6.09 (2H, m), 5.67 (1H, s), 5.05 (1H, dt, *J* = 13.7, 2.5 Hz), 2.57 (1H, ddd, *J* = 5.5, 14.4, 17.8 Hz), 2.46−2.42 (2H, m), 2.21 (1H, br t, *J* = 10.6 Hz), 1.96 (3H, s), 1.66 (3H, s), 1.63 (3H, s), 1.62 (3H, s), 1.12 (3H, s), 1.01 (3H, d, *J* = 6.8 Hz), 0.77 (3H, s). ^{13}C NMR (151 MHz) 199.5 (s), 170.4 (s), 163.8 (s), 141.2 (d), 127.9 (d), 126.7 (s), 123.8 (s), 123.6 (d), 75.0 (d), 53.1 (d), 52.8 (d), 50.7 (d), 43.7 (s), 39.5 (d), 39.5 (t), 37.7 (d), 36.0 (s), 33.9 (t), 33.9 (t), 32.1 (t), 27.2 (t), 23.8 (t), 21.3 (q), 20.7 (q), 20.6 (t), 20.4 (q), 18.9 (q), 16.2 (q), 13.0 (q), 11.8 (q). Anal. Calcd for $C_{30}H_{44}O_3$: C, 79.59; H, 9.80. Found C, 79.41; H 9.89.

(22*R*)-22-Acetoxyergosta-1,4,6,24-tetraen-3-one (32). Trienone **31** (363.1 mg, 0.80 mmol) was treated with 455.5 mg (2.00 mmol) of DDQ in 30 mL of benzene (see **29**) to give 281 mg of **32** (77% yield). R_f 0.56 (33% EtOAc−hexane). $[\alpha]^{22}_D$ −9.5° (*c* 0.58, CH_2Cl_2). ^{1}H NMR (600 MHz) 7.06 (1H, d, *J* = 10.1 Hz), 6.26−6.22 (2H, m), 6.04 (1H, d, *J* = 10.4 Hz), 6.00 (1H, s), 5.04 (1H, dt, *J* = 11.1, 2.4 Hz), 2.43 (1H, dd, *J* = 11.4, 13.9 Hz), 2.27 (1H, br t, *J* = 10.6 Hz), 1.97 (3H, s), 1.65 (3H, s), 1.62 (3H, s), 1.61 (3H, s), 1.19 (3H, s), 1.00 (3H, d, *J* = 6.8 Hz), 0.79 (3H, s). ^{13}C NMR (151 MHz) 186.5 (s), 170.5 (s), 162.8 (s), 153.0 (d), 138.5 (d), 128.1 (d), 127.6 (d), 126.8 (s), 123.8 (s), 123.7 (d), 74.9 (d), 53.2 (d), 52.7 (d), 48.24 (d), 43.3 (s), 41.2 (s), 39.5 (d), 39.4 (t), 38.1 (d), 32.0 (t), 27.1 (t), 23.8 (t), 21.8 (t), 21.3 (q), 20.8 (q), 20.7 (q), 20.5 (q), 18.9 (q), 13.0 (q), 12.0 (q). Anal. Calcd for $C_{30}H_{42}O_3$: C, 79.95; H, 9.39. Found: C, 79.77; H 9.21.

(22*R*)-22-Hydroxyergosta-1,4,6,24-tetraen-3-one (33). Acetate **32** (308 mg, 0.68 mmol) was treated with 0.5 g (3.6 mmol) of K_2CO_3 in 10 mL of MeOH at room temperature. After stirring overnight, the mixture was filtered, and the solvent was removed under reduced pressure. The residue was taken up with Et_2O and filtered though silica gel to give 280 mg (quant.) of pure alcohol **33**. R_f 0.38 (33% EtOAc−hexane). ^{1}H NMR (300 MHz) 7.05 (1H, d, *J* = 10.2 Hz), 6.25−6.19 (2H, m), 6.03 (1H, dd, *J* = 9.9, 1.8 Hz), 5.99 (1H, s), 3.76 (1H, dt, *J* = 11.1, 2.4 Hz), 2.39 (1H, dd, *J* = 11.3, 13.5 Hz), 2.26 (1H, br t, *J* = 10.3 Hz), 1.70 (6H, s), 1.65 (3H, s), 1.19 (3H, s), 0.99 (3H, d, *J* = 6.9 Hz), 0.82 (3H, s). ^{13}C NMR (75 MHz) 186.3 (s), 162.6 (s), 152.9 (d), 138.5 (d), 128.6 (d), 128.1 (s), 127.6 (d), 124.2 (s), 123.8 (d), 70.5 (d), 53.3 (d), 52.8 (d), 48.4 (d), 43.4 (s), 41.2 (s), 40.5 (d), 39.5 (t), 38.2 (d), 34.5 (t), 27.3 (t), 23.8 (t), 21.8 (t), 20.9 (q), 20.7 (q), 20.6 (q), 18.4 (q), 12.4(q), 12.0 (q). MS (EI) *m*/*z* (relative intensity) 409 (30, $M^+ + 1$), 325 (50), 171 (18), 84 (100). HRMS (EI) *m*/*z* 408.3028. (calcd for $C_{28}H_{40}O_2$, 408.3036).

(22*R*,24*S*)-22-Acetoxy-24,25-epoxyergosta-1,4,6-triene-3-one (34). To 180 mg (0.44 mmol) of **33** in 15 mL of dry benzene was added 20 mg (75 μmol) of vanadyl acetylacetonate. After stirring 5 min at room temperature, the resulting green solution was treated with 100 μL (0.5−0.6 mmol) of 5−6 M *tert*-butyl hydroperoxide/decane. After another 10 min, the reaction was quenched with 1 mL of pyridine/Ac_2O 2:1 and allowed to stand room temperature overnight. After evaporation of solvents under reduced pressure, the product was purified by flash chromatography (20% EtOAc−hexane) to give 189 mg of **34** (92% yield). R_f 0.25 (33% EtOAc−hexane). $[\alpha]^{20}_D$ −7.8° (*c* 0.54, CH_2Cl_2). ^{1}H NMR (300 MHz) 7.02 (1H, d, *J* = 10.2 Hz), 6.22−6.16 (2H, m), 5.99 (1H, d, *J* = 10.4 Hz), 5.95 (1H, s), 5.18 (1H, dt, *J* = 11.1, 2.4 Hz), 2.03 (3H, s), 1.29 (3H, s), 1.25 (3H, s), 1.24 (3H, s), 1.15 (3H, s), 0.91 (3H, d, *J* = 6.6 Hz), 0.75 (3H, s). ^{13}C NMR (75 MHz) 186.2 (s), 170.5 (s), 162.5 (s), 152.8 (d), 138.2 (d), 128.0 (d), 127.5 (d), 123.8 (s), 123.6 (d), 73.0 (d), 62.7 (s), 61.2 (s), 53.2 (d), 52.4 (d), 48.1 (d), 43.2 (s), 41.0 (s), 39.8 (d), 39.2 (t), 38.0 (d), 32.4 (t), 27.1 (t), 23.6 (t), 21.6 (t), 21.4 (q), 21.1 (q), 21.1 (q), 20.6 (q), 19.3 (q), 12.7 (q), 11.8 (q). MS (EI) *m*/*z* (relative intensity) 466 (4, M^+), 406 (6), 171(26),111(100). HRMS (EI) *m*/*z* 466.3083 (calcd for $C_{30}H_{42}O_4$, 466.3091).

(22*R*,24*R*)-Ergosta-1,4,6-trien-3-one 22,24,25-Orthoacetate (29) from 34. Epoxy acetate **34** (311 mg, 0.70 mmol) was treated with 35 mL of 10 mM TFA in dry benzene under N_2 at room temperature. After 4 h, the solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (5−20% EtOAc−hexane) to give 307 mg of **29** (99% yield).

(22*R*,24*R*)-6α,7α-Epoxyergosta-1,4-dien-3-one 22,24,25-Orthoacetate (35). A stirred solution of **29** (201 mg, 0.43 mmol) in 10 mL of CH_2Cl_2 was treated with 210 mg (0.91 mmol) of *m*-CPBA (75%) for 24 h at 0−4 °C. The reaction was quenched with 10 mL of a 5% NaOH and extracted with Et_2O. After being dried with Na_2SO_4, the solvent was removed under reduced pressure, and the product was purified by flash

chromatography (20−33% EtOAc−hexane) to give 202 mg of **35** (99% yield): R_f 0.30 (33% EtOAc−hexane). $[\alpha]^{20}_D$ +4.3° (*c* 0.09, CH_2Cl_2). ^{1}H NMR (300 MHz) 7.00 (1H, d, J = 10.2 Hz), 6.46 (1H, d, J = 1.8 Hz), 6.22 (1H, dd, J = 10.2, 1.8 Hz), 4.20 (1H, dt, J = 11.3, 4.4 Hz), 3.62 (1H, d, J = 3.6 Hz), 3.34 (1H, d, J = 2.0 Hz), 1.56 (3H, s), 1.31 (3H, s), 1.20 (3H, s), 1.19 (3H, s), 1.17 (3H, s), 0.97 (3H, d, J = 6.6 Hz), 0.78 (3H, s). ^{13}C NMR (75.5 MHz) 185.1 (s), 159.8 (s), 153.2 (d), 131.0 (d), 127.7 (d), 117.2 (s), 82.4 (s), 81.2 (s), 69.7 (d), 53.7 (d), 51.8 (d), 51.6 (d), 50.7 (d), 43.2 (s), 40.9 (s), 39.1 (t), 38.5 (d), 38.2 (d), 35.2 (d), 30.2 (t), 27.2 (t), 25.1 (q), 23.5 (t), 23.4 (q), 21.8 (t), 20.5 (q), 20.4 (q), 19.9 (q), 12.5 (q), 11.7 (q). Anal. Calcd for $C_{30}H_{42}O_5$: C 74.65; H 8.71. Found C 74.23; H 8.46.

(22*R*,24*R*)-7α-Hydroxyergosta-1,4-dien-3-one 22,24,25-Orthoacetate (Petuniasterone D, 1). Small pieces of aluminum foil (45 mg) were immersed in 2% $HgCl_2$ in H_2O for 15 s, washed with EtOH, and introduced to a solution of **35** (234 mg, 0.51 mmol) in 10 mL of THF/H_2O 9:1 at 0 °C. After 1.5 h, the black solids were filtered off, and the solution was extracted with Et_2O and H_2O. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the crude 7α-hydroxy-$\Delta^{1,5}$-3-ketone was isomerized, without isolation, with 1 g of K_2CO_3 in 15 mL of MeOH. After being stirred for 0.5 h at room temperature, the mixture was filtered, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (20−33% EtOAc−hexane) to give 213 mg of **1** (91% yield): R_f 0.40 (50% EtOAc−hexane); ^{1}H NMR (300 MHz) 7.06 (1H, d, J = 10.1 Hz), 6.26 (1H, dd, J = 10.1, 1.9 Hz), 6.14 (1H, t, J = 1.5 Hz), 4.19 (1H, dt, J = 11.4, 4.2 Hz), 4.03 (1H, br s), 2.75, (1H, ddd, J = 13.8, 3.1, 1.7 Hz), 2.47 (1H, dd, J = 13.8, 3.1 Hz), 1.56 (3H, s), 1.29 (3H, s), 1.24 (3H, s), 1.19 (3H, s), 1.17 (3H, s), 0.97 (3H, d, J = 6.6 Hz), 0.76 (3H, s). ^{13}C NMR (75 MHz) 185.4 (s), 164.1 (s), 155.3 (d), 127.6 (d), 127.2 (d), 117.2 (s), 82.4 (s), 81.2 (s), 69.7 (d), 69.5 (d), 52.0 (d), 49.9 (d), 44.4 (d), 43.3 (s), 42.8 (s), 40.8 (t), 39.7 (d), 38.9 (t), 38.5 (d), 30.2 (t), 27.1 (t), 25.1 (q), 23.7 (t), 23.4 (q), 22.4 (t), 20.4 (q), 19.8 (q), 18.1 (q), 12.5 (q), 11.7 (q).

Acknowledgment. Funding was provided by USDA-CSREES NRICGP grant number 99-35311-8272 and the Petroleum Research Fund, administered by the American Chemical Society. We thank Carl Elliger for the sample of authentic petuniasterone D and Peter G. M. Wuts (Pharmacia and Upjohn Company) for the gift of stigmasterol.

Supporting Information Available: Copies of ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO020068S