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Steroid Conformations in Solid and Solution: Stereoselectivity of Grignard Addition to 20-Keto Steroids

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Stereoselectivity of the Grignard addition to pregnenolone was studied by use of regiospecific isotope labeling in order to reconcile conflicting concepts for the conformational isomerism of the steroid side chain. (20*S*)- and (20*R*)-[20-methyl-labeled]-20-methyl-5-pregnene-3β,20-diols (**4a** and **4b**) were synthesized by addition of (a) CD_3MgI to pregnenolone acetate, (b) CH_3MgI to pregnenolone-17α,21,21,21-d_4, and (c) $^{13}CH_3MgI$ to pregnenolone acetate. Stereoselectivity of the Grignard addition was analyzed by proton NMR at 60 MHz in $CDCl_3$ [20(*pro-S*)-CH_3 at 72 Hz, 20(*pro-R*)-CH_3 at 79 Hz, $J_{^{13}C-H}$ = 126 Hz], and the ratio of 20*S* to 20*R* was observed in all cases to be 9:1. Ethyl-Grignard addition to pregnenolone also gave ca. 9:1 for the 20*S*/20*R* ratio. The results indicate that the rotational isomerism around the C(17)–C(20) bond of pregnenolone in solution highly favors the above-*D*-ring conformation of the carbonyl group, opposing the recent claim that pregnenolone exists in a 6:4 equilibrium of "cis" and "trans" conformers. The assignment of the 20*S* configuration to the major product of the Grignard addition to pregnenolone was confirmed by x-ray crystallography for the first time.

Conformational isomerism in steroid chemistry still remains enigmatic. Nes and Varkey[2] recently reported that pregnenolone exists in benzene/ether as a 6:4 equilibrium of cis and trans conformers, directly based on their observed ratio of 20-hydroxycholesterol to 20-hydroxyisocholesterol which was obtained by isohexyl-Grignard addition to pregnenolone acetate. The same reaction has been previously reported by Petrow and Stuart-Webb[3] and Mijares et al.[4] as giving only one product which was assigned to be 20*S*.[4,5] These two groups reported that in no case was there any evidence for the formation of more than one C(20) stereoisomer,[3] and the isolated compound was the only product formed during the condensation, although a careful search was made to isolate the 20*R* epimer.[4] The hypothesis of Rakhit and Engel[6] that there exists four preferred conformations of 20-keto steroids, A_1, A_2, B_1, and B_2 as designated, has been used[2,5] for rationalization of their conclusions, but without specific evidence.

We have recently found[7] a high stereoselectivity for methyl-Grignard addition to 17α-hydroxypregnenolone (99% 20*S* addition) and 16α,17α-epoxypregnenolone (93% 20*R* addition), which indicates that these 20-keto steroids exist in solution highly selectively in the preferred conformation found in the solid state, as determined by x-ray crystallography. Therefore, in this paper we decided to measure the stereoselectivity of methyl-Grignard addition to pregnenolone in order to clarify the conformational preference of 20-keto steroids in solution. Examination of the side-chain conformation in the solid state of 35 steroid structures[8] shows that the carbonyl oxygen is located above the D ring as depicted in **1a** (Figure 1) whether or not the structure has a hydroxy substituent at the neighboring 17α and/or 21 positions. An unusual 20-carbonyl conformation eclipsed with the C(13)–C(17) bond (**1b**) has been observed in 16β-bromo,[9] 16α,17α-epoxy,[10] and 16β-methyl[11] substituted structures. The preferred *si*-face attack by the Grignard reagent on conformation **1a** and the *re*-face attack on conformation **1b** are predicted to occur for steric reasons,[7] therefore giving a 20*S* configuration (**2a**, Figure 1, the incoming alkyl (R) being C(17) > R > C(21)) for the major product from the pregnenolone reaction. To distinguish and quantitatively assess the chemically like, paired methyl groups at C(20) of the methyl-Grignard reaction product, we have chosen three regiospecific labeling sets, (a) deuterated Grignard reagent and pregnenolone acetate, (b) Grignard reagent and deuterated pregnenolone, and (c) ^{13}C-labeled Grignard reagent and pregnenolone acetate, and ^1H NMR at 60 MHz for analysis of the stereoselectivity of the reaction. Ethyl-Grignard addition to pregnenolone was also analyzed.

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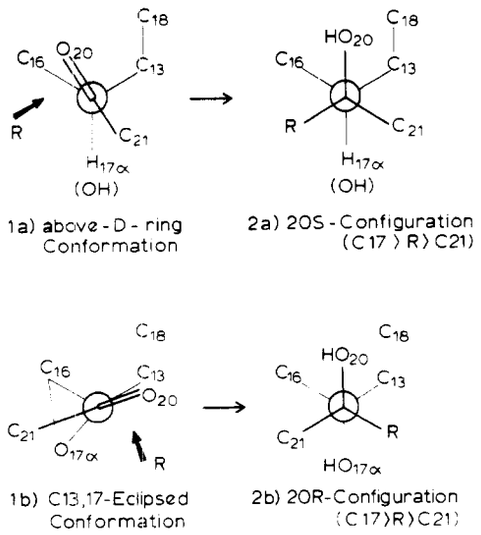


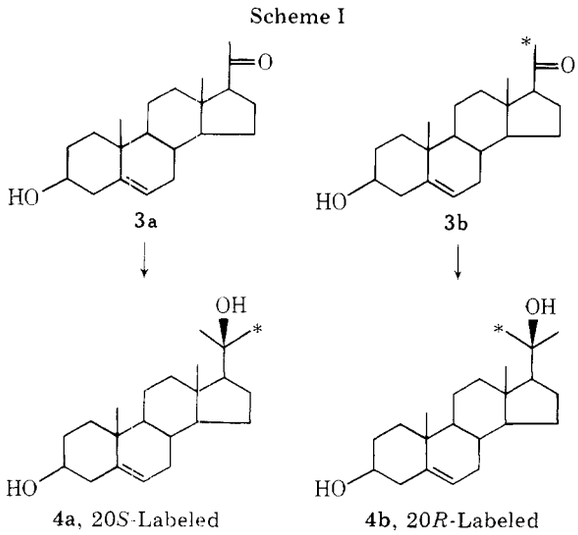
Figure 1. Conformations projected through C(20)–C(17). Preferred *si*-face attack by the Grignard reagent (R) is indicated for the conformation **1a** and *re*-face attack for **1b.**

Results and Discussion

20-Methyl-5-pregnene-3β,20-diol (**4**), mp 195–197 °C, was synthesized by addition of excess methylmagnesium bromide in ether to a benzene solution of pregnenolone (**3a;** Scheme I). The reaction was practically quantitative, and the purified product was isolated in 91% yield. ^{1}H NMR (Figure 2a) of **4** 3-acetate, mp 149–150 °C, showed two signals of equal intensity for the chemically like, paired methyl groups at 1.20 and 1.32 ppm. Deuterated methyl-Grignard reagent (99.5 atom % d_3) in 2 equiv was reacted with pregnenolone acetate (**3a** 3-acetate), and the product was isolated in a similar manner. ^{1}H NMR of (20*S*)-[20-C^{2}H$_3$]-**4a** 3-acetate showed a diminished peak for the 1.20-ppm signal while maintaining a nearly quantitative methyl signal at 1.32 ppm, as shown in Figure 2b. Quantitative analysis by the weight method in the expanded scan showed 88:12 for the 20*S*/20*R* ratio. If this analysis of the relative peak areas, which lie in the high-background area of skeletal protons, is reasonably accurate, the reversed deuterium labeling should show an inversed ratio for the 20*S*/20*R*. Therefore, pregnenolone-*17α,21,21,21-d*$_4$ (**3b,** Scheme I) was prepared by enolization in deuterated water with perchloric acid. ^{1}H NMR showed a disappearance of the C(21)–methyl signal, and **3b** was oxidized with Jones' reagent followed by an alkaline treatment to give progesterone-*17α,21,21,21-d*$_4$. Mass spectrometry of the product showed the labels to be 88 atom % at each position (Figure 3). After Grignard addition and acetylation, ^{1}H NMR of (20*R*)-[20-C^{2}H$_3$,17α-^{2}H]-**4b** 3-acetate showed, as expected, only a small peak at 1.32 ppm while maintaining approximately the full scale of methyl signal at 1.20 ppm (Figure 2c). Quantitative analysis by the weight method showed 13:87 for the 20*S*/20*R* ratio.

Even though it is evident from the deuterium-label study that methyl-Grignard addition is highly stereoselective, quantitative assessment of the selectivity is not very reliable in this area due to the unresolved high-background protons of the steroid skeleton. Thus, we have carried out a further ^{13}C-labeled Grignard reaction. We have previously applied[7] this to obtain a more accurate ratio by taking advantage of the large ^{13}C–^{1}H coupling constant of 126 Hz. In addition, tritium tracer was added to the reagent for two reasons: first, further to support our evidence[12] that there is no separation among rotational isomers around the C(17)–C(20) bond due to purification procedures; second, to use the radioisotopically labeled product as a substrate to study the stereomechanism of steroid biosynthesis. The methyl-Grignard reaction gave a mixture of labeled products which showed only one radioisotopic spot on TLC, and the radioactive mixture showed no indication of separation through the purification proce-

Scheme I



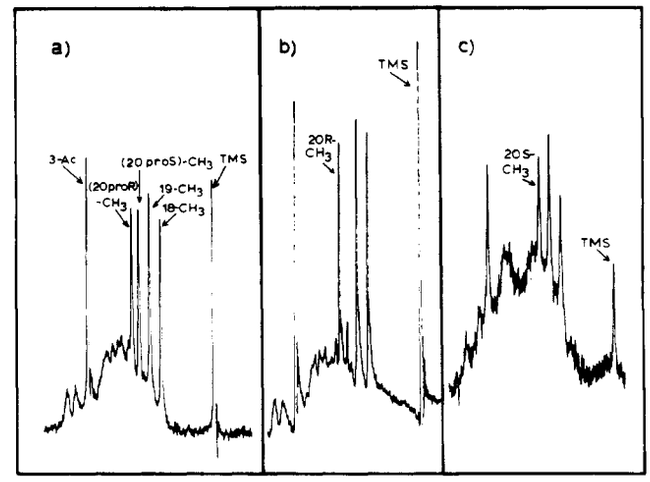


Figure 2. ^{1}H NMR spectra: (a) 20-methyl-5-pregnene-3β,20-diol 3-acetate (**4** 3-Ac), (b) (20*S*)-[^{2}H-labeled]**4a** 3-Ac, and (c) (20*R*)-[^{2}H-labeled]**4b** 3-Ac. All spectra were measured in deuteriochloroform at 60 MHz using tetramethysilane (Me_4Si) as internal standard.

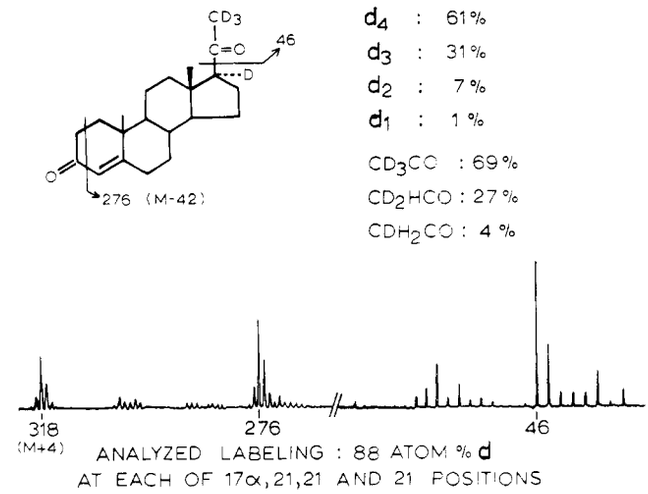


Figure 3. Mass spectrum of progesterone-*17,21,21,21-d*$_4$.

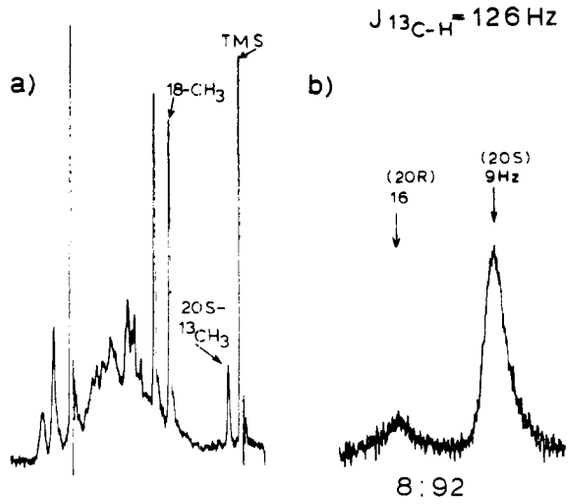


Figure 4. ^1H NMR spectra: (a) (20*S*)-[20-$^{13}CH_3$]-20-methyl-5-pregnene-3β,20-diol 3-acetate and (b) an expanded spectra (1 ppm full scale) of the same compound.

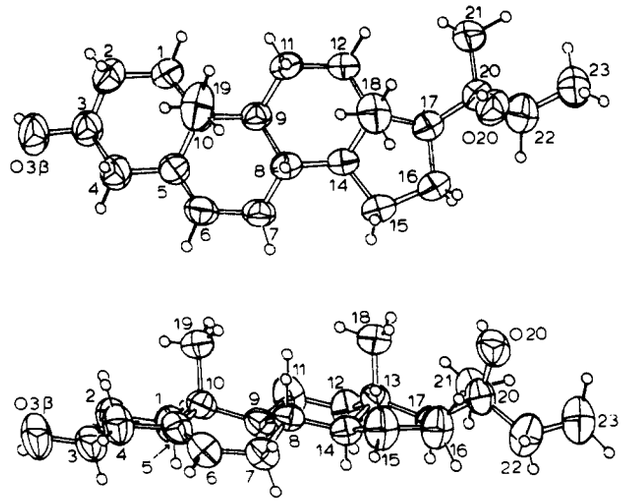


Figure 5. Observed conformation of (20*S*)-20-ethyl-5-pregnene-3β,20-diol. The thermal ellipsoids are scaled to a 50% probability level, and the hydrogens are shown as circles.

dures. ^1H NMR of (20*S*)-[20-$^{13}CH_3$-C^3H_3]-**4a** 3-acetate showed (Figure 4a) a predominant peak (approximately 1.5 H) at 0.15 ppm compared to an almost negligible signal at 0.27 ppm. In the expanded scan (Figure 4b), the stereoselectivity was analyzed to be 92:8 for the 20*S*/20*R* ratio. (20*S*)-[20-$^{13}CH_3$-C^3H_3]-20-Methyl-5-pregnene-3β,20-diol (92% 20*S*, 8% 20*R*) with a specific activity of 4.12 mCi of ^3H/mmol was obtained.

It is clear through the three sets of analyses that methyl-Grignard addition to pregnenolone has a 9:1 stereoselectivity. We had previously observed[13] 9:1 for 19*S*/19*R* in the sodium borodeuteride reduction of the 19-aldehyde of 17-benzoyloxy-3-oxo-4-androsten-19-al, where the "over A-ring" attack is clearly preferable to the "over C-ring" attack by the reagent and 2:1 for 19*S*/19*R* in the same reaction for 3β-hydroxy-17-oxo-5-androsten-19-al, where the steric preference of attack is not conspicuous when analyzed on the conformations determined[14,15] by x-ray crystallography. The stereoselectivity as measured by the product ratio depends not only on the equilibrium of the conformational isomers but also on the "α-side/β-side" attack ratio which is affected by each reagent–carbonyl conformer relationship. One cannot, therefore, attribute a priori an observed stereoselectivity to either or

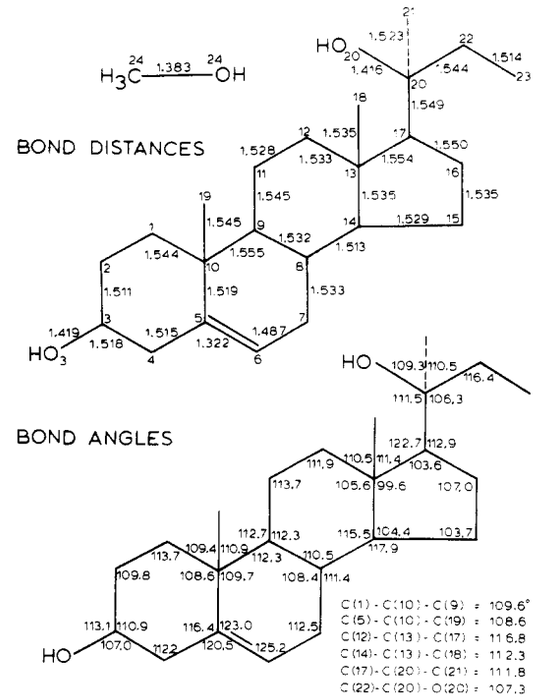


Figure 6. Observed bond lengths and angles in (20*S*)-20-ethyl-5-pregnene-3β,20-diol.

both of the factors quantitatively. However, in view of the facts that the methyl-Grignard reaction to 17α-hydroxypregnenolone and 16α,17α-epoxypregnenolone showed such highly selective addition to the opposite chirality and that the addition to pregnenolone showed again a high stereoselectivity, it is reasonable to assume that the steroid conformation in solution highly favors the same preferred conformation as found in the solid state.

The ethyl-Grignard reaction with pregnenolone was also carried out to assess the stereoselectivity of addition and to confirm the absolute configuration at C(20) by x-ray crystallography. The product showed characteristics similar to those previously reported.[16] The ratio of the 21-methyl signals at 1.25 ppm (20*S*) and 1.11 ppm (20*R*) of 20-ethyl-5-pregnene-3β,20-diol 3-acetate was 9:1.

The assignment of 20*S* for the absolute configuration of 20-alkyl-20-hydroxy steroids formed by the Grignard addition to pregnenolone has been made on the basis of the α-side attack and supported by a series of chemical modifications where steric control approach is assumed.[4,5] However, in view of our experience[13,17] for the necessary reassignment of the absolute configuration at C(19) of 19-alkyl-19-hydroxy steroids in spite of "the well-founded" chemical modification method,[18] we undertook the single-crystal growth and the total structural determination by diffraction methods of 20-ethyl-5-pregnene-3β,20-diol.

X-ray crystallographic analysis of the major diastereoisomeric product obtained from the ethyl-Grignard reaction shows conclusively that the configuration of C(20) is *S* (Figure 5). The crystals of (20*S*)-20-ethyl-5-pregnene-3β,20-diol were obtained from a methanol solution and contain one molecule of methanol per steroid molecule. The bond distances and bond angles shown in Figure 6 are within the range of values normally found in other 5-pregnene molecules.[19] The flexible B ring in this structure has nearly ideal C(8)/C(9) half-chair conformation as indicated by the $\Delta C_2^{5,6}$ asymmetry parameter[20] value of 1.0°. The A and C rings both have chair conformations, and the D ring has a distorted conformation nearly midway between a C(13) β-envelope (ΔC_s^{13} = 11.0°) and a C(13)/C(14) half-chair (ΔC_2^{16} = 9.4°).

The pertinent parts of the molecular structure of the major diastereoisomer are the *S* configuration of C(20) and the conformation of the C(20) substituents relative to the D ring. Figure 7 shows the conformation of the C(17)–C(20) bond with 0(20) + gauche to C(13) and the ethyl substituent trans to C(13). Thus, the previous configurational assignment of 20-alkyl-20-hydroxy steroids is confirmed while that for 20-alkyl-17α,20-dihydroxy steroids still remains controversial.[5,7,21]

Shimizu in 1964 postulated[21] the 20*S* configuration for the isolated product of isohexyl-Grignard addition to 17α-hydroxypregnenolone acetate by simple assumption of the α-side attack, thus assigning the product to be 17α,20-dihydroxycholesterol. Chaudhuri et al. in 1969 reversed[5] the assignment to be 20*R* by chemical derivatization methods and thereby corrected the structure to be 17α,20-dihydroxyisocholesterol. We have in 1976 assigned[7] the 20*S* configuration for the isotope-labeled methyl-Grignard addition product of 17α-hydroxypregnenolone acetate by a correlation approach[13] of steroid conformations in solid and solution. If the stereochemical courses of the methyl- and isohexyl-Grignard reactions are assumed to be similar, it would indicate that the initial designation by Shimizu was correct, and the latter reassignment should be reversed. On the other hand, a large variance in stereoselectivity due to alkyl radicals of the Grignard reagents has been reported[5] to occur to a 20-keto steroid, 16α,17-epoxypregnenolone acetate. Therefore, a more decisive study is required in order to reconcile the controversy on the absolute configuration at C(20).

The high stereoselectivity observed in this study and the structure determination by x-ray crystallography support the concept that the rotational isomerism of the pregnenolone side chain in solution lies highly selectively (≥90%) toward the above D-ring conformation.

Experimental Section

Materials and General Methods. Methyl-d_3 iodide (99.5 atom %) was purchased from ICN, methyl-^{13}C iodide (90 atom %) from Bio-Rad Lab, [^{3}H]methyl iodide (80 mCi/mmol) from NEN Corp., and deuterium oxide (99.8%) from Mallinckrodt Chem. Methyl- and ethylmagnesium bromide in ether were purchased from Ventron Corp., and precoated silica gel GF plates (Uniplates, Analteck, Inc.) were used for TLC. Melting points were measured on a Fischer-Jones melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 267 spectrophotometer in KBr pellets. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Varian EM-360 spectrometer at 60 MHz using tetramethylsilane (Me_4Si) as an internal standard. The 20*S*-CH_3/20*R*-CH_3 ratios were measured by the weight method. The area of corresponding signals on Xerox copies of the expanded spectra (0–1 ppm full scale, five repeated scans) was cut off and weighed on a Metler H20T balance. Mass spectra were recorded using a Dupont (CEC) 21-21-491 double-focusing mass spectrometer. Radioisotope scanning of TLC plates was made by a Packard 7201 radiochromatogram scanner.

20-Methyl-5-pregnene-3β,20-diol 3-Acetate (4 3-Acetate). To a stirred solution of 316 mg (1 mmol) of pregnenolone (**3a**) in 80 mL of benzene was added 14 mL (40 mmol) of 2.86 M methylmagnesium bromide in ether. The reaction mixture was stirred at room temperature for 18 h and then refluxed for 2 h. The reaction mixture was decomposed by dropwise addition of 60 mL of 20% ammonium chloride in an ice bath, and the product was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to give 342 mg of crude product, mp 182–185 °C. TLC analysis showed only one spot (R_f 0.41, $CHCl_3$/acetone 8:2) with no sign of residual starting material. The crude product was recrystallized from chloroform/methanol to give 302 mg of 20-methyl-5-pregnene-3β,20-diol (**4**): mp 195–197 °C (187 °C,[3] 194–195 °C[22]); IR 3320, 1445, 1373, 1025 cm^{-1}.

A 129-mg portion of diol **4** was dissolved in 2 mL of pyridine and 0.4 mL of acetic anhydride. The mixture was left at room temperature for 16 h and then poured into ice water. The precipitates were collected by filtration and dried in vacuo to give 132 mg of the 3-acetate, mp 142–151 °C. The crude acetate was recrystallized from ethanol, giving 79 mg of **4** 3-acetate: mp 149–150 °C (151–152 °C[23]); IR 3320, 1720, 1465, 1360, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, Figure 2a) δ 0.85 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.20 (3H, s, 20(*pro-S*)-CH_3), 1.32 (3H, s, 20(*pro-R*)-CH_3), 2.04 (3H, s, 3-OAc), 5.40 (1H, m, 6-H).

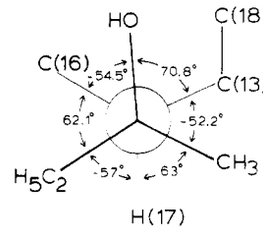


Figure 7. Observed conformation around the C(20)–C(17) bond of (20*S*)-20-ethyl-5-pregnene-3β,20-diol.

(20*S*)-[20-C^2H_3]-20-Methyl-5-pregnene-3β,20-diol 3-Acetate (4a 3-Acetate). An ethereal solution of methyl-d_3-magnesium iodide was prepared from 4 mmol of methyl-d_3 iodide and 4.25 mmol of magnesium turnings in 4 mL of anhydrous ether. The prepared reagent was added to 714 mg (2 mmol) of **3a** acetate in 20 mL of anhydrous benzene, and the mixture was stirred at room temperature for 16 h and then refluxed for 2 h. The mixture was decomposed by addition of 10 mL of 20% ammonium chloride solution, and the product was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give 722 mg of crude material, mp 122–137 °C. ^{1}H NMR of the crude material showed a signal ratio of 91:9 for δ 1.32 and 1.20 (20*S*/20*R*). A 621-mg portion of the product was recrystallized from benzene/hexane to give 449 mg of (20*S*)-[20-C^2H_3]-20-methyl-5-pregnene-3β,20-diol 3-acetate (**4a** 3-acetate): mp 141–145 °C; ^{1}H NMR ($CDCl_3$, Figure 2b) δ 0.85 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.32 (ca. 3H, s, 20*R*-CH_3), 2.04 (3H, s, 3-OAc), 5.40 (1H, m, 6-H). The signal ratio for δ 1.32 and 1.20 was 88:12 (20*S*/20*R*).

Pregnenolone-*17,21,21,21-d*$_4$ (3b). To a solution of 620 mg (1.96 mmol) of pregnenolone (**3a**) in 10 mL of dioxane was added 2 mL (100 mmol) of deuterated water and 0.11 mL of 70% perchloric acid, and the mixture was kept at 65 °C for 3 days. The solvent was evaporated under a nitrogen stream, and the residue was recrystallized from methanol to give 169 mg of **3b**, mp 192–193 °C. The nearly quantitative labeling at C(21) was shown by the disappearance of the methyl signal at 2.1 ppm. A 120-mg portion of **3b** in 20 mL of acetone was treated with Jones' reagent (0.1 mL of standard solution[24]), and the resultant unconjugated ketone was treated with 0.03 mL of 5% NaOH in 15 mL of methanol. The product was purified through preparative TLC and recrystallizations to give 32 mg of progesterone. The deuterium labeling at C(21) was shown by ^{1}H NMR to again be extensive, and it was quantitatively analyzed by mass spectrometry. The areas of *m/e* 318 (M + 4), 276 (M + 4 − 42), and 46 (C^2H_3CO) are shown in Figure 3, and the labeling is calculated to be 88 atom % at each of the 17α,21,21, and 21 positions.

(20*R*)-[20-C^2H_3, 17α-^{2}H]-20-Methyl-5-pregnene-3β,20-diol 3-Acetate, (4b 3-Acetate). To a solution of 16 mg (0.05 mmol) of pregnenolone-*17α,21,21,21-d*$_4$ (**3b**) in 20 mL of benzene was added 10 mmol of methylmagnesium bromide (2.86 M in ether). The mixture was stirred at room temperature for 16 h and then decomposed with 15 mL of 20% ammonium chloride solution. The product was extracted with methylene chloride, and the extract was washed with water, dried with anhydrous sodium sulfate, and evaporated to dryness. The residue was acetylated with 1.0 mL of pyridine and 0.2 mL of acetic anhydride at room temperature for 16 h. Pouring into ice water and separation by filtration gave 15 mg of **4b** 3-acetate. The total crude material was used for ^{1}H NMR measurements; ($CDCl_3$, Figure 2c) δ 0.85 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.20 (ca. 3H, s, 20*S*-CH_3), 2.04 (3H, s, 3-OAc), 5.40 (1H, m, 6-H). The signal ratio for δ 1.32 and 1.20 was 13:87 (20*S*/20*R*).

(20*S*)-[20-$^{13}CH_3$-C^3H_3]-20-Methyl-5-pregnene-3β,20-diol 3-Acetate (4a 3-Acetate). An ethereal solution of 1.75 mmol of methyl-^{13}C iodide traced with [^{3}H]methyl iodide (12.5 mCi) was added to 48 mg (1.9 mmol) of ether-washed magnesium turnings. After a vigorous reaction subsided, the mixture was refluxed for 15 min. To a solution of 360 mg (1 mmol) of **3a** acetate in 8 mL of benzene was added 1 mmol of the methylmagnesium iodide solution (0.4 mL) prepared above. The mixture was stirred at room temperature for 16 h, refluxed for 1 h, and decomposed with 5 mL of 20% ammonium chloride solution. The product was extracted with methylene chloride, and the extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give 376 mg of crude material. The crude product showed 99% of the total radioisotope in a single

peak at R_f 0.60 (chloroform/acetone 8:2) superimposed with authentic 20-methyl-5-pregene-3β,20-diol 3-acetate in the radioisotope scanning of the TLC plate. Some amount of unreacted pregnenolone acetate was detected in the crude material by TLC, IR, and ^{1}H NMR. Half of the product (188 mg) was subjected to preparative TLC (chloroform/acetone 8:2 followed by chloroform/acetone 95:5), and the radioactivity was detected by exposure to an x-ray film (Kodak RP/R2) which showed only one band. The radioisotopic band was eluted with ethanol/ether (2:8) and evaporation of the eluent gave 74 mg of (20*S*)-[20-$^{13}CH_3$-C^3H_3]-20-methyl-5-pregnene-3β,20-diol 3-acetate (**4a** acetate): mp 146–151 °C; ^{1}H NMR ($CDCl_3$, Figure 4) δ 0.15 and 2.25 (ca. 1.5 H each, d, (20*S*)-20-$^{13}CH_3$, $J_{H-^{13}C}$ = 126 Hz), 0.27 (ca. 0.1H, (20*R*)-20-$^{13}CH_3$, $J_{H-^{13}C}$ = 126 Hz), 0.87 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.05 (3H, s, 3-OAc), 5.45 (1H, m, 6-H). The signal ratio for δ 0.15 and 0.27 was 92:8 (20*S*/20*R*).

(20*S*)-[20-$^{13}CH_3$-C^3H_3]-20-Methyl-5-pregnene-3β,20-diol (4a). A solution of 22 mg of **4a** 3-acetate in 2 mL of ether was added to a suspension of 168 mg of lithium aluminum hydride in 25 mL of ether, and the mixture was stirred at room temperature for 30 min and then refluxed for 1 h. Water (12 mL) and 1 N H_2SO_4 (12 mL) was added, and the product was extracted with ether. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give 24 mg of residue. The crude product was recrystallized from chloroform/methanol to give 12 mg of **4a:** mp 196–197 °C; specific activity, 4.12 mCi of ^{3}H/mmol.

20-Ethyl-5-pregnene-3β,20-diol 3-Acetate. To a solution of 1 g (3.18 mmol) of **3a** in 170 mL of benzene was added 26 mL (78 mmol) of 3 M ethylmagnesium bromide in ether. The mixture was stirred at room temperature for 17 h and then refluxed for 1 h. After treating with 120 mL of 20% ammonium chloride solution, the product was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was recrystallized from methylene chloride/methanol to give 903 mg of 20-ethyl-5-pregene-3β,20-diol: mp 159–165 °C; IR 3350, 1460, 1375, 1190, 1060, 1025 cm^{-1}. A 208-mg portion of the diol was acetylated with 2 mL of pyridine and 0.4 mL of acetic anhydride at room temperature for 16 h. The mixture was poured into ice water, and the precipitates were collected by filtration, washed with water, and dried in vacuo, giving 208 mg of crude material, mp 165–169 °C. The product was recrystallized from methanol to give 126 mg of 20-ethyl-5-pregnene-3β,20-diol 3-acetate: mp 171–175 °C (178–181 °C);[16] IR 3500, 1720, 1460, 1370, 1250, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, s, 18-CH_3), 1.03 (3 H, s, 19-CH_3), 1.11[16] (ca. 0.3H, s, (20*R*)-20-CH_3), 1.25 (ca. 3 H, s, (20*S*)-21-CH_3), 2.04 (3 H, s, 3-OAc), 5.38 (1 H, m, 6-H). The relative intensity of the signals at δ 1.25 and 1.11 was 9:1 (20*S*/20*R*).

X-Ray Crystallography. X-ray crystallographic analysis of (20*S*)-20-ethyl-5-pregnene-3β,20-diol/methanol (1:1) was carried out using a crystal with dimensions 0.36 × 0.40 × 0.41 mm obtained by evaporation of an acetone/methanol solution. The crystal data are as follows: $C_{23}H_{38}O_2 + CH_3OH$, formula wt 378.60 g; monoclinic; a = 13.7409 (7), b = 7.5992 (7), c = 11.0979 (8) Å, β = 104.857 (5)°; V = 1120.10 Å^3; Z = 2; ρ_{obsd} = 1.129 g cm^{-3}, ρ_{calcd} = 1.123 g cm^{-3}; space group $P2_1$.

The data were measured on a Nonius CAD-4 automatic diffractometer using Ni-filtered Cu Kα̅ radiation (λ = 1.54178 Å) to a maximum θ of 75° at room temperature. A total of 2496 independent data were measured, of which 2124 had net intensities of at least twice the estimated standard deviation and were considered observed. The structure was solved by the multisolution direct methods program MULTAN[25] and refined by a full-matrix least-squares procedure to a final R value ($\Sigma||F_o| - |F_c||/\Sigma|F_o|$) of 4.6% for the observed data and 5.6% for all data. The positional and thermal parameters for all atoms except the y coordinate of C(9) were allowed to vary in the final cycles of refinement. The final fractional coordinates are given in Table I (Supplementary Material). Listings of F_o and F_c may be obtained from the authors.

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Registry No.—3a, 145-13-1; **3a** acetate, 1778-02-5; **3b,** 61574-54-7; **4,** 20976-92-5; **4** 3-acetate, 64070-60-6; [20-$^{13}CH_3$-C^3H_3]-**4a,** 64070-55-9; [20-$^{13}CH_3$]-**4a** 3-acetate, 64070-61-7; [20-C^2H_3]-**4a** 3-acetate, 64070-57-1; [20-C^2H_3,17α-^{2}H]-**4a** 3-acetate, 64070-59-3; [20-$^{13}CH_3$-C^3H_3]-**4a** 3-acetate, 64082-21-9; [20-C^2H_3,17α-^{2}H]-**4b** 3-acetate, 64082-22-0; [20-^{2}H]-**4b** 3-acetate, 64070-58-2; [20-$^{13}CH_3$-C^3H_3]-**4b** 3-acetate, 64070-56-0; methyl bromide, 74-83-9; deuterated water, 7789-20-0; [^{3}H]methyl iodide, 50630-93-8; (20*S*)-ethyl-5-pregnene-3β,20-diol 3-acetate, 21902-59-0; (20*R*)-ethyl-5-pregnene-3β,20-diol 3-acetate, 21902-60-3; (20*S*)-ethyl-5-pregnene-3β,20-diol, 54082-55-2; 20*R*-ethyl-5-pregnene-3β,20-diol, 23071-01-4; acetic anhydride, 108-24-7; (20*S*)-ethyl-5-pregnene-3β-20-diol: methanol (1:1), 64070-54-8; progesterone-17α,*21,21,21-d*$_4$, 64070-62-8; methyl-^{13}C iodide, 4227-956.

Supplementary Material Available: A listing of the atomic and fractional coordinates with the thermal parameters (2 pages). Ordering information is given on any current masthead page.

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