Synthesis of [19-$^2H_3$]Progesterone and [18-$^2H_3$]Progesterone

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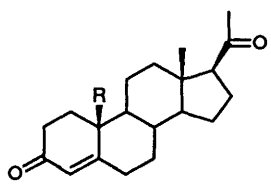
[19-$^2H_3$]Progesterone has been prepared from 3,3:20,20-bisethylenedioxy[19-$^2H_3$]pregn-5-en-19-ol by reduction of the derived 19-toluene-*p*-sulphonate with lithium triethylborodeuteride ('superdeuteride') followed by hydrolysis of the ethylenedioxy groups. [18-$^2H_3$]Progesterone was obtained from (20*R*)-3β-acetoxy-pregn-5-eno-20,18-lactone *via* conversion into methyl (20*R*)-3β-20-dihydroxypregn-5-en-18-oate: a two-stage introduction of three atoms of deuterium at C-18 *via* the toluene-*p*-sulphonate of the derived [18-$^2H_2$]-18-ol required unusual experimental conditions.

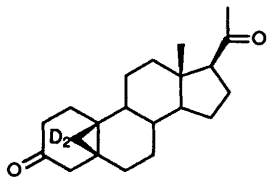
Progesterone labelled with three or four deuterium atoms at chemically stable sites was required initially as a mass spectrometric standard for isotope-dilution studies, and more recently in connection with an investigation into the peripheral metabolism of progesterone in the ovary, placenta,[1] or kidney[2] to give 21-hydroxypregn-4-ene-3,20-dione (11-deoxycorticosterone), a metabolite known to contribute to hypertension in certain clinical situations.

We have already described[3] a multi-step synthesis of [11,11,12,12-$^2H_4$]progesterone from the steroidal sapogenin hecogenin. We now report syntheses of the two trideuteriated forms of progesterone named in the title.

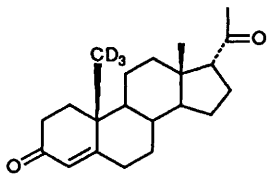
[19-$^2H_3$]*Progesterone* (**4**).—A first and somewhat unsatisfactory synthesis was based upon the previously reported procedure[4] for the preparation of [19-$^2H_3$]androst-4-ene-3,17-dione. 19-Hydroxy-[19-$^2H_2$]pregn-4-ene-3,20-dione (**1**)[5] was converted into its 19-toluene-*p*-sulphonate (**2**), which was reduced with zinc dust in aqueous acetic acid to give 5,19-cyclo-5β-[19-$^2H_2$]pregnane-3,20-dione (**3**). The final deuterium atom was introduced at C-19, with cleavage of the cyclopropane ring, by heating of the 5,19-cyclo compound (**3**) with deuteriated hydrochloric acid [^{2}HCl–^2H_2O]. On a small scale, this reaction proceeded reasonably well without any organic co-solvent, as described for androstenedione.[4] However, larger batches (of the order of 1 g) failed to mix well with the ^{2}HCl–^2H_2O so that the reaction became inefficient. Moreover, and more seriously, the resulting [19-$^2H_3$]progesterone (**4**) was heavily contaminated by its 17α-isomer (**5**), formed *via* enolisation of the 20-oxo group under the strongly acidic conditions. This problem is peculiar to the pregnan-20-one series, and had no counterpart in the reported [19-$^2H_3$]androstenedione synthesis. Separation of progesterone from 17-isoprogesterone on the required scale called for repeated and tedious chromatography. This method of synthesis was therefore unattractive.



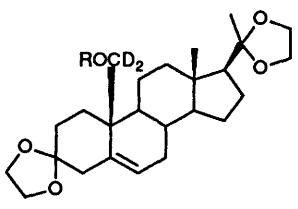
(**1**) R = HOD_2C
(**2**) R = $TsOD_2C$
(**4**) R = CD_3



(**3**)



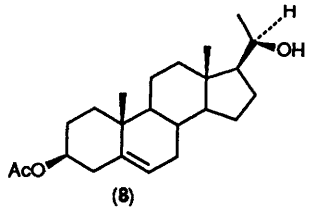
(**5**)



(**6**) R = H
(**7**) R = Ts

We have now devised a modified procedure from 3,3;20,20-bisethylenedioxy-[19-$^2H_2$]pregn-5-en-19-ol (**6**), prepared as previously described.[5] The derived 19-toluene-*p*-sulphonate (**7**) merely reverted to the 19-hydroxy compound (**6**), by reduction at sulphur, when treated with lithium aluminium deuteride in common solvents. We found, however, that lithium triethylborodeuteride ['superdeuteride'] was able to replace the toluene-*p*-sulphonyloxy group by deuterium, to give [19-$^2H_3$]progesterone (**4**) after mild acidic hydrolysis of the protecting acetal groups. Although a comparable yield of deuteriated 5,19-cyclo-5β-pregnane-3,20-dione [*cf.* structure (**3**)] was also obtained from the products of superdeuteride reduction, the two compounds were readily separated by chromatography and there was no complicating isomerisation at C-17. This route to [19-$^2H_3$]progesterone therefore has clear advantage over that used originally, although the deuterium content (see Experimental section) was lower than expected. The reason for this is not clear. The location of a third deuterium atom in the 5β,19-cyclo compound obtained in this way has not been established with certainty; it is probably at the 6β position, by analogy with known solvolytic reactions of 19-sulphonates, giving 6β-substituted 5,19-cyclosteroids.[6]

[18-$^2H_3$]*Progesterone*.—Early attempts to obtain [18-$^2H_3$]-progesterone began with the preparation of (20*R*)-3β-acetoxy-pregn-5-eno-20,18-lactone (**9**) from (20*R*)-3β-acetoxypregn-5-en-20-ol (**8**), following a published procedure.[7] However, we were unable to achieve the reported yield (*ca* 40% overall) despite several attempts. It was next necessary to devise a reductive route for the stepwise introduction of three atoms of deuterium at C-18. With the C-3 oxygen function protected as its tetrahydropyranyl ether (**10**), reduction of the lactone with lithium aluminium deuteride gave the corresponding (20*R*)-[18-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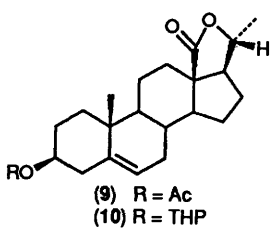
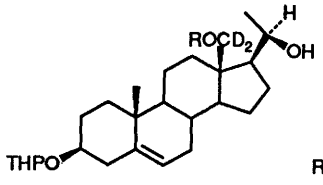
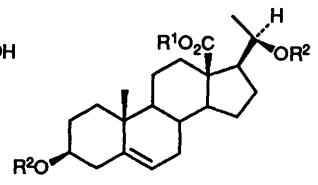
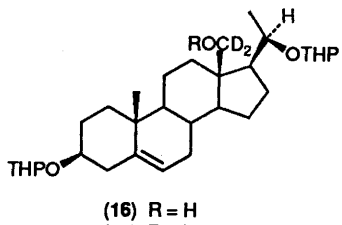
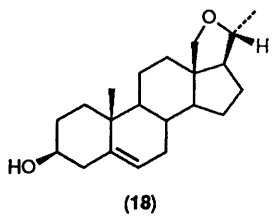

(9) R = Ac
(10) R = THP


(11) R = H
(12) R = Bu^{t}Me$_2$Si


(13) $R^1 = R^2 = H$
(14) R^1 = Me, R^2 = H
(15) R^1 = Me, R^2 = THP


(16) R = H
(17) R = Ts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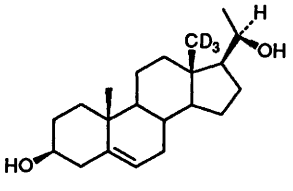
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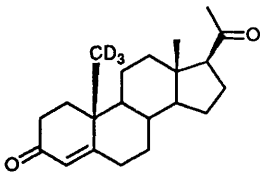
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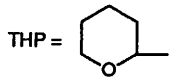

$^2H_2$]pregnane-18,20-diol derivative (**11**). Direct blocking of the 20-hydroxy group to permit selective transformations at C-18 could not be achieved, but the more reactive primary 18-hydroxy group was found to react selectively with t-butyldimethylsilyl chloride (TBDMSCl) under forcing conditions, to afford the corresponding 18-silyl ether (**12**),[8,9] permitting subsequent esterification of the 20-hydroxy group. However, all attempts at selective removal of the 18-silyl group, by reaction with fluoride ion,[8–10] then failed to give the required 20-monoester of the 18,20-diol. Products formed under a variety of conditions were invariably complex and intractable mixtures. No other protective reagent specific for the 18-hydroxy group could be found.

A possible alternative strategy involved controlled reduction of the lactone (**10**) with di-isobutylaluminium deuteride (DIBAL-D). Model experiments with the commercially available hydride reagent (DIBAL) gave the 20,18-lactol which could be easily converted by hydroxylamine into the 20-hydroxy-18-aldoxime. However, the corresponding reaction of the lactol with hydrazine was slow and inefficient; an attempted Wolff–Kishner reduction by heating of the lactol with an excess of hydrazine and sodium hydroxide in diethylene glycol gave (20*R*)-pregn-5-ene-3β,20-diol in such low yield that the corresponding sequence from the lactone with deuteriated reagents was not attempted. The foregoing abortive experiments are not reported in detail.

These difficulties were finally circumvented by subjecting the lactone (**9**) to vigorous alkaline hydrolysis:[11] careful acidification then gave the labile 20-hydroxy-18-oic acid (**13**) which was directly methylated (diazomethane) to give the known methyl ester (**14**).[11] Both hydroxy groups were then protected by forming the 3,20-bis(tetrahydropyran-2-yl) ether (**15**), and the 18-ester was reduced with lithium aluminium deuteride to give the [18-$^2H_2$]pregnan-18-ol derivative (**16**), which was converted into its 18-toluene-*p*-sulphonate (**17**). Attempts to follow our successful procedure as for reduction of the 19-tosylester (**7**) were unsuccessful. 'Superdeuteride' merely reduced the 18-tosyl ester (**17**) at sulphur, giving the 18-ol (**16**). Lithium aluminium deuteride, in refluxing tetrahydrofuran (THF) or 1,4-dioxane, was also ineffective.

We eventually overcame this difficulty by using an uncommon reducing system comprising a stirred suspension of lithium aluminium deuteride in refluxing hexane. Two products were obtained. The less polar was the (20*R*)-[$^2H_2$]-18,20-epoxide (**18**), but the major product, after removal of the protecting tetrahydropyranyl ether groups, was the required (20*R*)-[18-$^2H_3$]pregn-5-ene-3β,20-diol (**19**). It is not clear why only the suspended reagent carries out the desired reduction: reaction occurs presumably at the surface of the solid. Benzene could be substituted for hexane, but refluxing toluene led to the 18,20-epoxide (**18**) and recovered 18-ol (**16**) as major products.

A final Oppenauer oxidation, supplemented by further oxidation of some of the half-oxidised product (20*R*)-20β-hydroxy-[18-$^2H_3$]pregn-4-en-3-one with Jones' chromic acid reagent, gave [18-$^2H_3$]progesterone (**20**).

The whole sequence leading to [18-$^2H_3$]progesterone is significantly shorter than that for either the [19-$^2H_3$] or the previously described[3] [11,11,12,12-$^2H_4$] compound, and gave the most satisfactory deuterium content. It would offer even greater advantage if the reported yield in the early steps leading to the lactone (**9**) could be reproduced.

Experimental

M.p.s were determined with a Reichert hot-stage apparatus and are uncorrected. NMR spectra were determined for solutions in $CDCl_3$ unless specified otherwise, with either a Bruker WH400 (University of London Intercollegiate Research Service) or a Bruker AM 250 spectrometer. Chemical shifts are relative to internal tetramethylsilane; primed locants refer to tetrahydropyranyl protons. *J*-Values were estimated by measurement of signal splittings. Mass spectra were obtained with a Kratos MS 902 operating at 70 eV. Deuterium analyses were by mass spectrometry with direct insertion of the samples. IR spectra were determined for KBr discs. Preparative medium-pressure chromatography was carried out using Merck Kieselgel 60-HPF. All solvents were distilled before use. 'Light petroleum' refers to the fraction boiling in the range 60–80 °C. Hexane and THF were distilled from sodium. Pyridine was distilled from CaH_2. Lithium aluminium deuteride (98% ^2H) was obtained from Fluka. Lithium triethylborodeuteride ('superdeuteride') was obtained from Aldrich.

3,3:20,20-*Bisethylenedioxy*-19-(p-*tolylsulphonyloxy*)-[19-$^2H_2$]*pregn*-5-*ene* (**7**).—A solution of 3,3:20,20-bisethylenedioxy[19-$^2H_2$]pregn-5-en-19-ol (**6**) (1.3 g, 3.1 mmol) in pyridine (20 ml), stirred at *ca.* 0–5 °C, was treated with toluene-*p*-sulphonyl chloride (1.4 g, 7.5 mmol). The solution was allowed to warm to room temperature and was stirred for 4 days. The reaction was then quenched by pouring the solution onto crushed ice (*ca.* 600 ml) and the mixture was stirred for 1 h.

The precipitate was collected, washed, and dried under reduced pressure at room temperature (*ca.* 12 h) and then at 40 °C (*ca.* 24 h) to afford crude 3,3:20,20-bisethylenedioxy-19-(*p*-tolylsulphonyloxy)-[19-$^2H_2$]pregn-5-ene (**7**) (1.3 g, 74%), which was reduced without further purification, as described below.

[19-$^2H_3$]*Pregn-4-ene-3,20-dione* (**4**).—The crude 19-toluene-*p*-sulphonyl ester (**7**) (800 mg, 1.4 mmol), prepared as above, was added to a solution of lithium triethylborodeuteride ('superdeuteride'; 50 ml; 1.0M in THF) under rigorously dry conditions. The solution was heated under reflux for 2 h, then cooled (ice-bath) and treated dropwise with water (*ca.* 10 ml) until gas evolution ceased. The resulting cooled suspension (ice-bath) was then treated carefully with aq. NaOH (42 ml; 3M) followed by H_2O_2 (42 ml; 30%) and allowed to warm to room temperature (1 h). The THF was separated and the aqueous layer was extracted with light petroleum (3 × 60 ml). The combined organic layers were washed with water (3 × 60 ml) and dried ($MgSO_4$). Evaporation of the solvents gave a syrup (780 mg), which was dissolved in aq. 80% acetone (50 ml) and the mixture was heated under reflux with toluene-*p*-sulphonic acid (PTSA) (a few crystals) for *ca.* 3 h. The solvent was then evaporated off (<40 °C) under reduced pressure, the residue was taken up with ethyl acetate (*ca.* 20 ml), and the solution was washed successively with saturated aq. $NaHCO_3$ (2 × 10 ml) and water (3 × 10 ml), and then dried ($MgSO_4$). Evaporation of the solvent gave a syrup, which was purified by medium-pressure chromatography on silica gel (60-H, PF) with light petroleum–ethyl acetate (3:2) as eluant. Two main fractions were collected. The first gave the 5,19-*cyclo*-5β-*pregnane*-3,20-*dione* (**3**) (170 mg, 38.6%), m.p. 126–131 °C; ν_{max} 2 180 (C–D) and 1 710 cm^{-1} (C=O); *m/z* (M^+), 317.2432 ($C_{21}H_{27}D_3O_2$ requires *M*, 317.2431) (M^+, 100%, 73% $^2H_3$), 316 (M^+, 20, 14% $^2H_2$), 315 (M^+, 7.5, 3.4% $^2H_1$), and 314 (M^+, 12.5, 9.6% $^2H_0$); δ 0.63 (3 H, s, 18-H_3), 2.13 (3 H, s, 21-H_3), 2.53 (2 H, apparent d, *J* 2 Hz, 4-H_2), and 2.57 (1 H, t, *J* 10 Hz, 17-H).

The second fraction afforded [19-$^2H_3$]pregn-4-ene-3,20-dione (**4**) (155 mg, 35.7%), m.p. 122–124 °C (from light petroleum) (lit.,[11] 121 °C or 128 °C); λ_{max}(MeOH) 240 nm (ε 18 800); $[\alpha]_D^{24}$ +192° (*c* 0.26, $CHCl_3$) (lit.,[11] +202°, unlabelled); ν_{max} 2 240 (C–D), 1 705 (C=O), 1 670 (conj. C=O), and 1 620 cm^{-1} (C=C); *m/z* 317 (M^+, 89%, 79% $^2H_3$), 316 (M^+, 7, 5% $^2H_2$), 315 (M^+, 8, 5% $^2H_1$), 314 (M^+, 12.7, 11% $^2H_0$), 275 [(M − C_2H_2O)$^+$; 52.7], 232 [(M − C_5H_9O)$^+$; loss of C-15, -16, -17, -20, and -21; 29], 191 (M − $C_8H_{11}O$; probably loss of ring D, 13-CH_3, and side-chain, 24), and 127 (100); δ(400 MHz) 0.67 (3 H, s, 18-H_3), 0.98 (1 H, td, *J* 11, 11, and 4 Hz, 9-H), 1.07 (1 H, m, $w_{\frac{1}{2}}$ 43 Hz, 7α-H), 1.19 (1 H, m, $w_{\frac{1}{2}}$ 40 Hz, 14-H), 1.28 (1 H, m, $w_{\frac{1}{2}}$ 36 Hz, 15β-H), 1.46 (2 H, m, $w_{\frac{1}{2}}$ 24 Hz, 11β- and 12α-H), 1.57 (1 H, qd, *J* 11, 11, 11, and 3.5 Hz, 8-H), 1.72 (4 H, complex, $w_{\frac{1}{2}}$ 55 Hz, 1α-, 11α-, 15α-, and 16α-H), 1.88 (1 H, m, $w_{\frac{1}{2}}$ 24 Hz, 7β-H), 2.04 (1 H, ddd, *J* 13, 5, and 3 Hz, 1β-H), 2.07 (1 H, dd, *J* 8 and 3 Hz, 12β-H), 2.13 (3 H, s, 21-H_3), 2.20 (1 H, m, $w_{\frac{1}{2}}$ 32 Hz, 16β-H), 2.37 (4 H, complex, $w_{\frac{1}{2}}$ 84 Hz, 2- and 6-H), 2.55 (1 H, t, *J* 8.7 Hz, 17-H), and 5.74 (1 H, s, 4-H).

Methyl (20R)-3β,20-*Dihydroxypregn-5-en*-18-*oate* (**14**).—In a modification of the published procedure,[12] (20*R*)-3β-acetoxypregn-5-eno-20,18-lactone (**9**) (3.9 g, 10.5 mmol) and sodium methoxide (8.0 g, 150 mmol) in 98% aq. butan-2-ol (120 ml) were heated under reflux for 2 h. The 18-carboxylic acid (**13**) was isolated essentially as described[12] (3.5 g, 97%); ν_{max} 3 420br (between 3 600 and 2 400; OH and CO_2H), and 1 695 cm^{-1} (C=O); δ 0.94 (3 H, s, 19-H_3), 1.15 (3 H, d, $J_{20.21}$ 6 Hz, 21-H_3), 3.48 (1 H, m, $w_{\frac{1}{2}}$ 27 Hz, 3-H), 3.61 (1 H, m, $w_{\frac{1}{2}}$ 20 Hz, 20-H), and 5.34 (1 H, m, $w_{\frac{1}{2}}$ 10 Hz, 6-H).

The crude acid (**13**), with diazomethane,[12] gave the methyl ester (**14**) (7.4 g, 88.6%), m.p. 183–184 °C (from acetone–light petroleum) (lit.,[12] 182–184 °C); ν_{max} 3 500 and 3 380 (O–H) and 1 702 cm^{-1} (CO_2Me); *m/z* 362 (M^+, 46%), 344 [(M − H_2O)$^+$, 48], 330 [(M − C_2H_2O)$^+$, 78], and 312 (100); δ(400 MHz) 0.90 (3 H, s, 19-H_3), 1.14 (3 H, d, $J_{20.21}$ 6.2 Hz, 21-H_3), 2.21 (1 H, br t, $J_{gem} \approx J_{3\alpha.4\beta}$ 13 Hz, 4β-H), 2.29 (1 H, dd, J_{gem} 13, $J_{3\alpha.4\alpha}$ 4 Hz, 4α-H), 2.66 (1 H, dt, J_{gem} 13, $J_{11\alpha.12\beta} = J_{11\beta.12\beta}$ = 3 Hz, 12β-H), 3.51 (1 H, m, $w_{\frac{1}{2}}$ 32 Hz, 3-H), 3.73 (3 H, s, CO_2Me), and 5.30 (1 H, m, $w_{\frac{1}{2}}$ 10 Hz, 6-H).

Methyl (20R)-3β,20-*Bis*(*tetrahydropyran*-2-*yloxy*)*pregn*-5-*en*-18-*oate* (**15**).—2,3-Dihydropyran (25 ml, 280 mmol, distilled from sodium) was added, dropwise, to a stirred solution of diol (**14**) (3.7 g, 10.1 mmol) and PTSA (*ca.* 8 mg) in THF (*ca.* 100 ml) at room temperature. The mixture was stirred for 24 h and the resulting solution was then diluted with diethyl ether (*ca.* 350 ml), and then washed successively with saturated aq. $NaHCO_3$ (2 × 70 ml) and water (2 × 70 ml), dried (Na_2SO_4), and concentrated under reduced pressure to give a yellow syrup, which was crystallised from aq. ethanol to give pale yellow crystals (4.78 g; dried at 40 °C under reduced pressure for 2 days, 90%). This material was a mixture of diastereoisomers and was used without further purification; ν_{max} 1 710 (CO_2Me) and 1 610 cm^{-1} (C=C); δ(400 MHz) 0.90 (1.5 H, s) and 0.92 (1.5 H, s) (19-H_3), 1.08 (1.5 H, d, $H_{20.21}$ 6.2 Hz) and 1.22 (1.5 H, d, $J_{20.21}$ 6.2 Hz) (21-H_3), 1.54 (12 H, m, $w_{\frac{1}{2}}$ 22 Hz, THP), 3.33 (0.5 H, m, $w_{\frac{1}{2}}$ 24 Hz), and 3.75 (0.5 H, m, $w_{\frac{1}{2}}$ 25 Hz) (20-H), 3.49 (3 H, m, $w_{\frac{1}{2}}$ 25 Hz, 6′-H and 3-H), 3.64 (1.5 H, s), and 3.65 (1.5 H, s) (CO_2Me), 3.90 (2 H, m, $w_{\frac{1}{2}}$ 25 Hz, 6′-H), 4.53 (0.3 H, m, $w_{\frac{1}{2}}$ 10 Hz), and 4.72 (0.7 H, m, $w_{\frac{1}{2}}$ 11 Hz) (2′-H), and 5.34 (1 H, m, $w_{\frac{1}{2}}$ 11 Hz, 6-H).

(20R)-3β-*Bis*(*tetrahydropyran*-2-*yloxy*)-[18-$^2H_2$]*pregn*-5-*en*-18-*ol* (**16**).—A solution of the methyl ester (**15**) (4.7 g, 8.9 mmol) in THF (50 ml) was added dropwise to a stirred suspension of lithium aluminium deuteride (1.5 g, 35 mmol) in THF (200 ml) at room temperature. The stirred suspension was heated under reflux for 20 h, then cooled (ice-bath), and the excess of lithium aluminium deuteride was destroyed by dropwise addition of THF–water (4:1). After 1 h at room temperature the flocculent precipitate was removed by filtration through Celite and washed thoroughly with THF (*ca.* 200 ml). The combined filtate and washings were concentrated to dryness to give a syrup, which was crystallised from methanol to afford the 18-ol as white crystals (2.5 g). The remainder of the product was recovered by medium-pressure chromatography, with silica gel (60-H, TLC grade), and light petroleum–ethyl acetate mixtures as eluant to give additional 18-ol (1.5 g; total yield 4.0 g, 90%). This material was a mixture of diastereoisomers and was used without separation; ν_{max} 3 480 (O–H), 2 210 and 2 120 cm^{-1} (C–D); *m/z* 402 (M^+ − 102, 11%, *ca.* 98% $^2H_2$) and 85 (C_5H_9O, 100); δ 1.03 (3 H, s, 19-H_3), 1.15 (*ca.* 2 H, d, $J_{20.21}$ 6.2 Hz), and 1.40 (*ca.* 1 H, d, $J_{20.21}$ 6.2 Hz) (21-H_3), 3.51 (3 H, m, $w_{\frac{1}{2}}$ 25 Hz, 6′-H), 3.95 (3 H, m, $w_{\frac{1}{2}}$ 42 Hz, 3-, 20-, and 6′-H), 4.55 (0.6 H, m, $w_{\frac{1}{2}}$ 11 Hz), and 4.72 (1.4 H, m, $w_{\frac{1}{2}}$ 10 Hz) (2′-H), and 5.34 (1 H, m, $w_{\frac{1}{2}}$ 11 Hz, 6-H); ^2H NMR (38.4 Hz) 3.52 (br s, 18-$^2H_2$).

(20R)-3β,20-*Bis*(*tetrahydropyran*-2-*yloxy*)-18-(p-*tolylsulphonyloxy*)[18-$^2H_2$]*pregn*-5-*ene* (**17**).—A mixture of the crude 18-ol (**16**) prepared as above (2.5 g, 5 mmol) in pyridine (40 ml) was stirred and treated with toluene-*p*-sulphonyl chloride (3.1 g, 16 mmol) and a few crystals of 4-dimethylaminopyridine at room temperature for 6 days, then was poured onto crushed ice (*ca.* 800 ml) and left for 1 h. The precipitate was separated by filtration and washed thoroughly with water to remove pyridine, and gave the solid 18-toluene-*p*-sulphonate ester (**17**) (3.12 g, dried over P_2O_5 under reduced pressure) as a mixture of diastereoisomers which was not purified but was reduced directly (see below); ν_{max} 2 364 and

2 343 cm^{-1} (C–D); δ[$(CD_3)_2CO$] 0.68 (2.2 H, s) and 0.86 (0.8 H, s) (19-H_3), 1.06 (0.8 H, d, $J_{20,21}$ 6.2 Hz) and 1.19 (2.2 H, d, $J_{20,21}$ 6.2 Hz) (21-H_3), 2.45 (0.8 H, s) and 2.48 (2.2 H, s), (Ar*Me*), 3.84 (2 H, m, $w_{\frac{1}{2}}$ 30 Hz, 3- and 20-H), 4.54 (0.5 H, m, $w_{\frac{1}{2}}$ 12 Hz) and 4.73 (1.5 H, m, $w_{\frac{1}{2}}$ 12 Hz) (2′-H), 5.30 (1 H, m, $w_{\frac{1}{2}}$ 13 Hz, 6-H), 7.51 (2 H, m, $w_{\frac{1}{2}}$ 17 Hz, ArH), and 7.84 (2 H, m, $w_{\frac{1}{2}}$ 17 Hz, ArH).

(20R)-[18-$^2H_3$]*Pregn-5-ene*-3β,20-*diol* (**19**).—The crude [18-$^2H_2$]-labelled toluene-*p*-sulphonate (**17**) prepared above (2.2 g, 3.5 mmol) was added in one portion to a well stirred suspension of lithium aluminium deuteride (1.32 g, 31 mmol; 99% ^2H) in hexane (22 ml, distilled from sodium). The resulting slurry was heated at 85–88 °C and efficiently stirred for 20 h, then cooled (ice-bath), and the excess of lithium aluminium deuteride was destroyed by careful addition of THF–water (50 ml; 4:1). After 1 h at room temperature, the flocculent precipitate was removed by filtration through Celite and washed thoroughly with more THF (*ca.* 200 ml). The combined filtrate and washings were concentrated to dryness at <40 °C under reduced pressure to give a white solid, which was not purified but was stirred directly with 0.3% HCl in acetone–water (*ca.* 30 ml; 4:1) at room temperature for *ca.* 8 h. The acidic solution was evaporated at <40 °C to give crystalline material, which was purified by medium-pressure chromatography on silica gel. Elution with light petroleum–ethyl acetate mixtures (2:1 and 1:1) gave two fractions: the first fraction was (20*R*)-18,20-*epoxy*-[18-$^2H_2$]*pregn-5-en*-3β-*ol* (**18**) (320 mg, 28%); ν_{max} 2 360 and 2 340 cm^{-1} (C–D); m.p. 164–166 °C and 177–179 °C; *m/z* 318.2525 (M^+, 100%, 97.2% $^2H_2$) ($C_{21}H_{30}D_2O_2$ requires *M*, 318.2526), 317 (M^+, 2.3, 1.8% $^2H_1$), and 316 (M^+, 1.0, 0.9% $^2H_0$); δ(400 MHz) 0.95 (3 H, s, 18-H_3), 1.21 (3 H, dd, $J_{17,20}$ 1, $J_{20,21}$ 6.5 Hz, 21-H_3), 3.49 (1 H, m, $w_{\frac{1}{2}}$ 30 Hz, 3-H), 3.77 (1 H, m, $w_{\frac{1}{2}}$ 18 Hz, 20-H), and 3.34 (1 H, m, $w_{\frac{1}{2}}$ 10 Hz, 6-H); ^2H NMR (38.4 MHz) δ 3.48 (br s, 18-^2H) and 3.72 (br s, 18-^2H).

The second fraction was the required (20*R*)-[18-$^2H_3$]*pregn*-5-*ene*-3β,20-*diol* (**19**) (385 mg, 34%), m.p. 205–207 °C (lit.,[13] 199–200 or 201.5–203.5 °C; lit.,[14] 211–211.5 °C); ν_{max} 3 419 (O–H), 2 219 (C–D), 1 636 (C=C) and 1 057 cm^{-1} (C–O); *m/z* 321 (M^+, 100%, 97% $^2H_3$), 320 (M^+, 2.9, 1.3% $^2H_2$), and 318 (M^+, 1.8, 1.6% $^2H_0$); δ 1.03 (3 H, s, 19-H_3), 1.09 (3 H, d, $J_{20,21}$ 6 Hz, 21-H_3), 3.19 (1 H, m, $w_{\frac{1}{2}}$ 30 Hz, 3-H), 3.63 (1 H, m, $w_{\frac{1}{2}}$ 24 Hz, 20-H), and 5.31 (1 H, m, $w_{\frac{1}{2}}$ 10 Hz, 6-H).

[18-$^2H_3$]*Pregn*-4-*ene*-3,20-*dione* (**20**).—A solution of the diol (**19**) (1.5 g, 4.6 mmol) in toluene (200 ml, distilled from sodium) was treated with cyclohexanone (10 ml, freshly distilled). Some toluene was distilled off in a Dean–Stark apparatus until the distillate was clear. A solution of aluminium isopropoxide (20 ml; 1.0M in toluene) was then added gradually with distillation of toluene to maintain the volume. The mixture was heated for a further 1 h under reflux, then more cyclohexanone (10 ml) and aluminium isopropoxide solution (20 ml) were added gradually and the mixture was heated overnight. The resulting suspension was then steam-distilled until the distillate appeared clear, and the residue was cooled, and extracted with diethyl ether (4 × 100 ml), and the combined extracts were dried (Na_2SO_4). Evaporation of the solvent gave a yellow syrup, which was purified by medium-pressure chromatography on silica gel with light petroleum–ethyl acetate mixtures (3:2 and 1:1) as eluant to give crystalline [18-$^2H_3$]pregn-4-ene-3,20-dione (**20**) (400 mg). A small amount (100 mg) of 20β-hydroxy-[18-$^2H_3$]pregn-4-en-3-one eluted subsequently was further oxidised with Jones' reagent (10 ml) in acetone in the usual way to give more [18-$^2H_3$]progesterone.

The product (**20**) was crystallised from light petroleum, m.p. 120–121 °C (lit.,[11] 121 °C or 128 °C); λ_{max}(MeOH) 240 nm (16 000); $[\alpha]_D^{25}$ +201° (*c* 0.21, $CHCl_3$) (lit.,[11] +202°, unlabelled); ν_{max} 2 230 (C–D), 1 705 (C=O), and cm^{-1} 1 665 (conj. C=O); *m/z* 317 (M^+, 82.6%, 92% $^2H_3$), 316 (M^+, 4.8, 5% $^2H_2$), 315 (M^+, 1.2, 0.7% $^2H_1$), 314 (M^+, 2.0, 1.8% $^2H_0$), 275 [$(M - C_2H_2O)^+$, 48.9], and 124 (100); δ(400 MHz) 0.98 (1 H, m, $w_{\frac{1}{2}}$ 28 Hz, 9-H), 1.19 (3 H, s, 19-H_3), 2.13 (3 H, s, 21-H_3), 2.54 (1 H, t, *J*, 8.7 Hz, 17-H), and 5.73 (1 H, s, 4-H); ^2H NMR (38.4 Hz) 0.6 (s, 18-$^2H_3$).

Unlabelled progesterone had *m/z* 314 (M^+, 22.7%), 272 (25), and 124 (100). The ^1H NMR spectrum (400 MHz) was identical with that of the [$^2H_3$]sample except for an additional singlet at δ 0.66 (3 H, 18-H_3).

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