Substituent Effects in ^{13}C Nuclear Magnetic Resonance Spectroscopy. Progesterone, Deoxycorticosterone, Corticosterone, Cortisol, and Related Steroids[1a]

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Abstract: The ^{13}C nmr spectra of the hormonal steroids progesterone, deoxycorticosterone, corticosterone, cortisol, as well as related monohydroxy- and dihydroxyprogesterone derivatives, have been obtained by the Fourier transform technique at 25.2 MHz. The signal due to each carbon atom has been assigned and some previous assignments in progesterone are altered. The spectra are analyzed in terms of substituent effects. Apart from the expected α, β, γ and δ effects, long range effects arising from conformational transmission through buttressing phenomena are observed. Shifts of about 0.1 ppm can be seen at certain carbon atoms resulting from the introduction of deuterium into the steroid. The conformation of the side chains in deoxycorticosterone is shown to involve orientation of the C(21) hydroxyl group toward the 17α substituent in DMSO solution.

Recently,[2,3] we attempted to relate changes in the chemical reactivity and biological activity of steroid hormones and their analogs to steric and electronic effects using X-ray diffraction studies and CNDO/2 calculations. Since the ^{13}C nmr (cmr) properties of organic molecules are markedly affected by steric and electronic changes brought about by the introduction of substituents, we have now used this method to examine some of the steroids we had investigated previously. Roberts[4] made the initial contribution in the cmr spectrometry of steroids with an analysis of the progesterone spectrum. In this paper we describe the reinvestigation of progesterone and the interpretation of cmr spectra of other hormonal steroids in the pregnane series. In a future paper we will relate these results to those obtained[1b] from 9α- and 6α-substituted analogs.

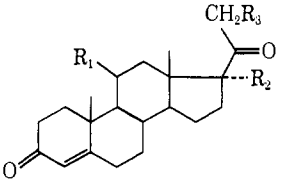
Experimental Section

Methods. Cmr spectra were obtained on a Varian XL-100-15 spectrometer using an S-124XL FT accessory with a 16K computer. Samples were spun in 8- or 12-mm tubes at approximately 30°. Samples were measured as 0.2–0.4 *M* solutions in DMSO-d_6 with TMS as the internal standard. Progesterone-d_9 required some added $CDCl_3$ to achieve solution. Proton noise decoupled (PND) spectra were obtained following 0.4–1.4 hr of accumulation. Single frequency off-center decoupled (SFOCD) (off-resonance) spectra were obtained by irradiating with a CW frequency at about δ-4 or +12 in the proton spectrum. In all cases the Fourier transform (FT) technique was applied.[5]

Progesterone, 11α-hydroxyprogesterone, 17α-hydroxyprogesterone, deoxycorticosterone, 11β,17α-dihydroxyprogesterone, corticosterone, and cortisol (Table I) were purchased. 11β-Hydroxyprogesterone was synthesized by the method of Magerlein and Levin[6] and purified by preparative tlc.

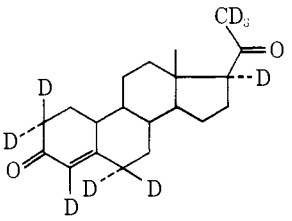
Progesterone (**4**) was deuterated smoothly and specifically at several positions to give progesterone-d_9 (**1**) by base-catalyzed exchange[7] in deuteriomethanol. The C-18 (0.65 ppm) and C-19 (1.19 ppm) pmr shifts remain unchanged upon deuteration of the

Table I. Progesterone and Hydroxylated Derivatives



Compound	R_1	R_2	R_3
Progesterone (**4**)[a]	H	H	H
Progesterone-d_9 (**1**)	H	H	H
11α-Hydroxyprogesterone (**5**)	OH[b]	H	H
11β-Hydroxyprogesterone (**6**)	OH	H	H
17α-Hydroxyprogesterone (**7**)	H	OH	H
Deoxycorticosterone (**8**)	H	H	OH
Corticosterone (**9**)	OH	H	OH
11β,17α-Dihydroxyprogesterone (**10**)	OH	OH	H
Cortexolone (**11**)	H	OH	OH
Cortisol (**12**)	OH	OH	OH

[a] The carbon-13 spectra of protio progesterone (**4**) were determined in DMSO-d_6 and dioxane. [b] R_1 in this compound is on the α side of the molecule and has an equatorial conformation.



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(1) (a) This investigation was supported in part by U. S. Public Health Service Research Grants AM-05016 and AM-14824 (to M. E. W.) and by Training Grant GM-00728 (D. D. G.). (b) Taken from the Ph.D. Thesis of D. D. G., University of California, San Francisco, 1973. (c) University of California. (d) Louisiana State University. (e) Varian Associates.

(2) C. M. Weeks, W. L. Duax, and M. E. Wolff, *J. Amer. Chem. Soc.*, **95**, 2865 (1973).

(3) P. A. Kollman, D. D. Giannini, W. L. Duax, S. Rothenberg, and M. E. Wolff, *ibid.*, **95**, 2869 (1973).

(4) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *ibid.*, **91**, 7445 (1969).

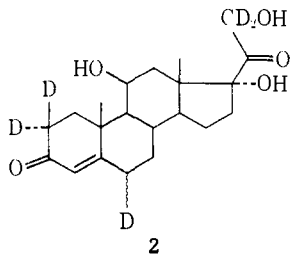
(5) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR," Academic Press, New York, N. Y., 1971.

(6) B. J. Magerlein and R. H. Levin, *J. Amer. Chem. Soc.*, **75**, 3654 (1953).

(7) L. Tökes and L. J. Throop in "Organic Reactions in Steroid Chemistry," J. Fried and J. A. Edwards, Ed., Van Nostrand-Reinhold, New York, N. Y., 1972, pp 152–153, and references cited therein.

other carbons. The absence of the pmr signals at 5.75 and 212 ppm clearly demonstrates that C-4 and C-21, respectively, are highly deuterated. Chemical ionization analysis indicated that the compound contained 15% d_9, 39% d_8, 30% d_7, and 15% d_6.

Considerable decomposition occurred when cortisol was heated in alkaline media in deuteriomethanol.[8] However, deuteration at room temperature during 69 hr using deuteriomethanol, dioxane, and deuterium oxide in the presence of sodium methoxide gave the desired **2**. Selective reduction of the 6,7 double bond of cortisol with deuterium gas and Pd/C provided a useful method for deuterium labeling of C-6 and C-7 (**3**).



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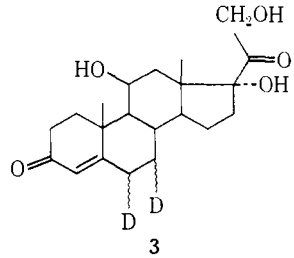

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Melting points were determined with a Thomas-Hoover apparatus (capillary tube) equipped with a corrected thermometer. Pmr spectra were obtained on a Varian A-60A spectrometer in deuteriopyridine solution using tetramethylsilane as the internal standard. Chemical shifts are reported in δ (ppm) values. Mass spectrometric analyses were performed by Mr. William Garland and Dr. Robert Weinkam on an MS-902 spectrometer modified to obtain chemical ionization spectra.

4-Pregnene-3,20-dione-*2,2,4,6,6,17α,21,21,21-d*$_9$ (1). To a solution of 1.0 g of progesterone in 30 ml of 99% methanol-d_1 under a nitrogen atmosphere was added 300 mg of sodium methoxide. An additional 5 ml of deuteriomethanol was added after the first 24 hr due to some evaporation of solvent. The reaction was conducted at 25° for 72 hr. After removal of the solvent *in vacuo* at 25° the resulting solid was taken up in ether and washed several times with water until neutral and dried with anhydrous $MgSO_4$. Evaporation of the solvent afforded 880 mg of colorless crystalline solid, mp 117–120°.

11β,17α,21-Trihydroxy-4-pregnene-3,20-dione-*2,2,6,21,21-d*$_5$ (Cortisol-*d*$_5$) (2). Cortisol (1.0 g) was dissolved in 35 ml of anhydrous dioxane and 10 ml of anhydrous 99% ethanol-d_1. After addition of 5.0 ml of deuterium oxide 99.6% *d* and 300 mg of sodium methoxide, the solution was stirred at 25° under nitrogen for 69 hr. A tlc analysis (65% EtOAc:35% hexane) showed a mixture of two compounds. The solvent was evaporated *in vacuo* at 25° affording a gummy residue which was taken up in a mixture of ether and ethyl acetate. The organic phase was washed with water, dried with anhydrous $MgSO_4$, and evaporated. Recrystallization from ethyl acetate afforded 325 mg of homogeneous solid (tlc analysis). Chemical ionization analysis showed that the 303 fragment (loss of side chain) of the steroid contains 10% d_4, 13% d_3, 29% d_2, 30% d_1, and 10% d_0. Proton nmr supports the structure for this compound. Both C-18 (1.18 ppm) and C-19 (1.58 ppm) chemical shifts in precursor and product are identical. The intensity of several peaks in the methylene envelope (2.1–2.5 ppm) diminished while the peaks assigned to the C-21 protons disappeared upon deuteration. The C-4 signal remained unchanged.

11β,17α,21-Trihydroxy-4-pregnene-3,20-dione-*6ξ,7ξ-d*$_2$ (3). A solution of 11β,17α,21-trihydroxy-4,6-pregnadiene-3,20-dione 21-acetate[9] in 30 ml of benzene–ethanol (1 : 1) was added to a suspension of 10% Pd/C in 30 ml of the same solvent mixture. The catalyst was prereduced before the addition of steroid solution with D_2 (99.7%). D_2 uptake (atmospheric pressure) was terminated after 65 ml was consumed. Analysis of the reaction by tlc showed that two products were formed. The polar component (major) had the same R_f value as an authentic sample of 11β,17α,21-trihydroxy-4-pregnene-3,20-dione 21-acetate (**3**). The catalyst was removed by filtration and the solvent evaporated. The 21-acetate group was hydrolyzed by dissolving the crude mixture in methanol followed by the addition of aqueous 10% K_2CO_3. After refluxing the reactants under a nitrogen atmosphere for 15 min, a tlc analysis indicated that the deuterio derivative of 11β,17α,21-trihydroxy-4-pregnene-3,20-dione (**3**) was present by comparison with a nondeuterated authentic sample. The solution was cooled to 25° and neutralized with 1.0 *N* HCl. The solvent was removed *in vacuo* and the semisolid was redissolved in ethyl acetate, washed with H_2O, and dried with anhydrous $MgSO_4$. Evaporation of the solvent afforded 745 mg of a two-component mixture. Purification of the desired polar component was achieved by preparative thin-layer chromatography. Approximately 190 mg was applied to each plate and developed three times with a 55% ethyl acetate:45% hexane system. Isolation of material from the polar fraction afforded 320 mg which was recrystallized from acetone–water. The sample was shown to contain 35% d_2, 42% d_1, and 23% d_0, by chemical ionization analysis.

Results

Spectral Assignments. A number of peaks (*e.g.*, C-3 carbonyl) could be assigned directly on the basis of chemical shift by comparison with other compounds. In other cases, the assignment could be narrowed to only a few possibilities (*e.g.*, C-8, C-9, C-11, and C-14 are the only doublets) by the use of off-resonance (single frequency off-center) decoupling (SFOCD). Following selective deuteration, the disappearance of specific signals due to quadrupole broadening, spin–spin splitting, and decreased NOE enhancement served to identify still other peaks.

Assignments described below are considered unambiguous, except in the following four pairs of carbon atoms, in which the assignments may be reversed: C-12 and C-16 of 17α-hydroxyprogesterone, C-7 and C-16 of 11β,17α-dihydroxyprogesterone, and C-2 and C-12, and C-6 and C-16 of cortexolone. None of these pairs is crucial to the assignment of other peaks in the respective spectra, nor are any of the conclusions drawn in the Discussion, which are based on differences of 3–5 ppm, affected by this situation. All assignments for progesterone and cortisol are considered to be certain. Although Roberts[4] analyzed the cmr spectrum of progesterone, we reassigned the peaks for C-2, C-6, C-7, C-8, C-12, C-16, and C-21 based on SFOCD and deuteration studies.

Chemical Shift Assignments. Seven carbon atoms in each steroid had relatively constant chemical shifts despite large structural changes and were assigned on this basis alone. Unsaturated ketone carbons absorb at higher field[10,11] than saturated ketone carbons, due to the delocalization of π electrons from the double bond (C(4)–C(5)) and both types of carbonyl carbons are predictably found in well-defined regions of the ^{13}C spectrum, namely, 197 ppm for C(3) type carbons and 207 ppm for C(20) carbons. C(4) and C(5) can be easily assigned[12] with C(5) being the most downfield of the pair and typically around 170 ppm (Table II). C(18) and C(19) are the most upfield signals in all the spectra and the two quartets are identified from the SFOCD spectra. C(18) and C(19) in progesterone were previously differentiated by Roberts and coworkers[4] and we used these assignments. Since the two signals are well separated (usually by 3–4 ppm) and the compounds investigated are structurally closely related, it is likely the positions for C(18) and C(19) do not cross in the spectra, even when structural modifications are made either in progesterone or hydro-

(8) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 2071 (1963).

(9) E. J. Agnello and G. D. Laubach, *J. Amer. Chem. Soc.*, **82**, 4293 (1960).

(10) J. B. Stothers and P. C. Lauterbur, *Can. J. Chem.*, **42**, 1563 (1964).

(11) D. H. Marr and H. B. Stothers, *ibid.*, **45**, 225 (1967).

(12) *Cf.* D. H. Marr and J. B. Stothers, *ibid.*, **43**, 596 (1965), and references cited therein.

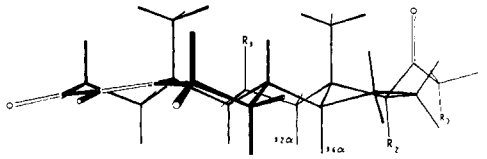

Figure 1. Dreiding model of substituted progesterone.

Table II. Carbon-13 Chemical Shifts and Assignments for Progesterone and Progesterone-d_9

Carbon	Progesterone[a]	Progesterone[b]	Progesterone-d_9[c]
1	35.1	36.3	35.2
2	33.5	34.2	
3	197.7	197.4	198.2
4	123.2	124.5	
5	170.6	169.5	170.4
6	31.9	32.9	
7	31.6	32.7	31.7
8	34.9	36.1	35.2
9	53.1	54.5	53.4
10	38.1	39.0	38.3
11	20.5	21.6	20.8
12	37.9	39.2	38.3
13	43.2	44.1	43.5
14	55.3	56.6	55.7
15	22.3	23.3	22.4
16	23.8	24.8	24.1
17	62.5	63.7	
18	12.9	13.3	13.1
19	16.8	17.3	17.2
20	208.2	207.4	208.4
21	31.0	31.0	

[a] Solvent, DMSO-d_6. [b] Solvent, dioxane. [c] Solvent, DMSO-d_6–$CDCl_3$.

cortisone. One of the most invariant triplets (21.8–23.3 ppm) in the SFOCD spectra is a signal at about 22.5 ppm which was assigned to C(15), following Roberts.[4] Although there are two other signals which flank C(15) at 23.8 and 20.5 ppm in progesterone, these two signals move 8.3 and 45.7 ppm in 17α-hydroxyprogesterone and 11β-hydroxyprogesterone, respectively, whereas the signal for C(15) remains almost the same.

Cortisol Assignments (Figure 2 and Table IV). C(3), C(4), C(5), C(15), C(18), C(19), and C(20) were assigned as above. Since the most downfield signals are C(20), C(3), C(5), and C(4), the next three upfield resonances are the alcohol carbons C-11, C-17, and C-21. From the SFOCD spectrum (not shown) the peaks 88.3 (singlet), 66.4 (doublet), and 65.8 (triplet) were assigned as C(17), C(11), and C(21), respectively.

The two remaining unassigned singlets at 38.8 and 46.2 ppm must be C(10) and C(13). C(13) should be affected by the removal of the 17α-OH group of hydrocortisone. In corticosterone (**9**) the 46.2-ppm resonance moves upfield by 3.0 ppm whereas the signal at 38.8 ppm remains unaffected. Thus, the 38.8-ppm signal is due to C(10) and the 46.2-ppm peak corresponds to C(13).

The three remaining doublets (55.5, 51.5, and 31.1 ppm) in the SFOCD spectrum of cortisol are C(9), C(14), and C(8). C(8) would be expected to have the most upfield resonance due to three 1,3-diaxial interactions with C(18), C(19), and 11β-OH, whereas the hydrogens on C(9) and C(14) have interactions only with other hydrogens. If the 11β-OH group were removed (cortexolone), C(8) would be the only nucleus of the three in which hydrogen would have decreased steric interaction. In cortexolone the *only* doublet which has moved downfield relative to cortisol is at 35.2 ppm (a 4.1-ppm downfield shift if 31.1 is C(8) in cortisol). The signal assigned to C(8) in cortexolone is invariant if no hydroxyl is present at C(11) (progesterone, deoxycorticosterone, and cortexolone). Thus, the signal at 31.1 is due to C(8). Differentiation of C(9) and C(14) can be made from the line shapes of the two remaining doublets. The doublet at 51.5 ppm is much broader than the one at 55.5 ppm. Since C(14) is coupled to more neighboring hydrogens, the signal due to C(14) would be broader; 51.5 is therefore C(14) and 55.5 is C(9).

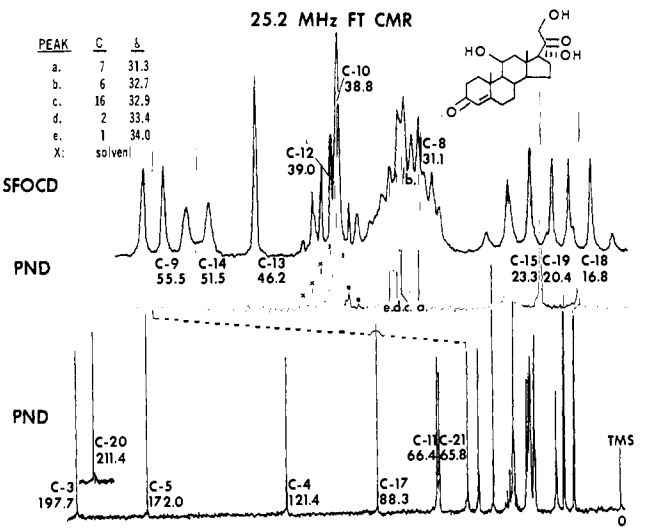

Figure 2. ^{13}C nmr spectrum and assignments (ppm) of cortisol.

The assignment of the remaining six carbons (triplets) is the most difficult, because some signals are separated by less than 0.5 ppm. Two deuterium-labeled cortisol compounds (**2** and **3**) were used to differentiate carbons C(2), C(6), and C(7). When cortisol was labeled with deuterium at C(6) and C(7), there was a diminution in the signal intensity at 31.3 and 32.7 ppm, whereas when cortisol was labeled at C(2) and C(6) there was a diminution in the signal intensity at 33.4 and 32.7. Since C(6) is common to both, the signal at 32.7 is C(6); 33.4 and 31.3 must be C(2) and C(7), respectively.

The two remaining unassigned carbons are C(1) and C(12). The triplet at 39.0 ppm has an upfield shift of 5.5 ppm when the 11β-OH is removed from hydrocortisone and must be C(12); the remaining signal thus is C(1) (34.0 ppm).

Progesterone Assignments (Figures 3 and 4 and Table III). Low solubility of cortisol and related compounds in other organic solvents necessitated the use of dimethyl sulfoxide (DMSO) for spectral studies. Since progesterone assignments had been reported in dioxane,[4] we determined assignments for progesterone in DMSO to eliminate solvent effects when comparing chemical shifts. Positions of the peaks for C(3), 3(4), C(5), C(18), C(19), and C(20) follow from the assignments of cortisol and are supported by the SFOCD spectrum. The C(21) signal is an easily recognized quartet centered at 31.0 ppm (previously[4] assigned to C(16)).

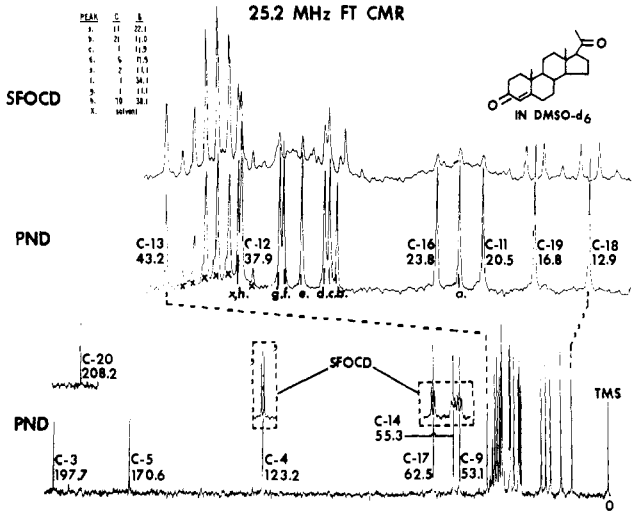

Figure 3. ^{13}C nmr spectrum and assignments (ppm) of progesterone.

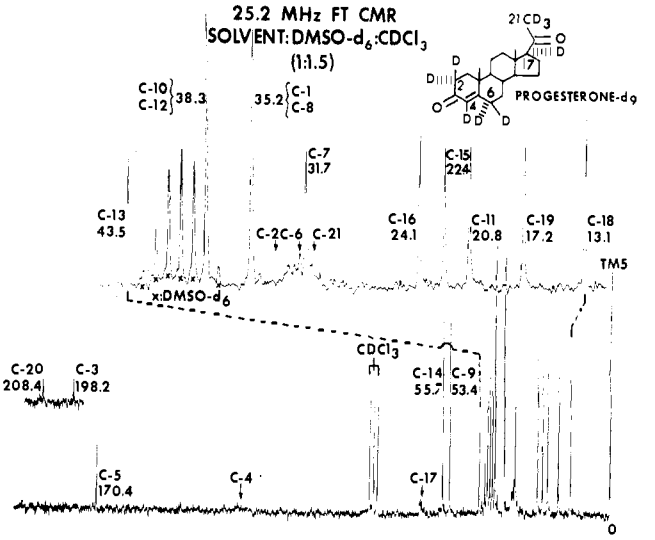

Figure 4. ^{13}C nmr spectrum and assignments (ppm) of progesterone-d_9.

The cmr spectrum of the 2,2,4,6,6,17α,21,21,21-d_9 derivative of progesterone (**1**) having five fewer resonances than the protio compound provides an independent assignment for these carbons. C(2) is readily differentiated from C(6) by comparison with hydrocortisone, and the triplets at 31.6 and 35.1 ppm are similarly assigned to C(7) and C(1), respectively.

The signal at 22.3 ppm is assigned to C(15) due to its positional invariance in the compounds studied. For the remaining three triplets, 20.5, 23.8, and 37.9 ppm, comparison with a monohydroxy compound is necessary. Thus, introduction of the 11β-hydroxyl causes the 20.5-ppm triplet to move downfield 45.7 ppm (11β-hydroxyprogesterone). The same hydroxyl group causes the C(12) triplet to move downfield by 8.9 ppm, while the 23.8 ppm triplet (C(16)) remains unchanged.

Due to the γ effect from C(18) and C(19), C(8) is expected at unusually high field; thus the 34.9-ppm signal is assigned to C(8). The same doublet is observed to move upfield 4.9 ppm upon introduction of the 11β-OH due to another 1,3-diaxial interaction. The broader of thh two remaining doublets is assigned to C(14) (55.3 ppm) because it is coupled to more hydrogens (at C(8) and C(15)) than C(9) (53.1) ppm.

C(10) and C(12) were the only signals which crossed upon changing solvents. Relative to DMSO C(12) moved downfield (1.3 ppm) in dioxane while C(10) moved upfield (0.9 ppm). Several other peaks moved slightly when the solvent was changed.

Monohydroxyprogesterone Assignments (Table III). The following carbons were assigned directly from the assignments in progesterone: C(1), C(2), C(3), C(4), C(5), C(6), C(7), C(10), C(13), C(15), C(18), C(19), and C(20).[13]

(a) 11α- and 11β-Hydroxyprogesterone (5 and 6). Having disposed of 13 signals, the remaining assignments are clear. C(14) (doublet), C(16) (triplet), C(17) (doublet), and C(21) (quartet) are readily identifiable from the SFOCD technique and comparison with progesterone. The assignment of C(14) for 11β-hydroxyprogesterone rests on the relatively broad doublet (56.8 ppm) observed in the SFOCD spectrum; as with progesterone the line shapes were useful for separating C(14) and C(9).

The two remaining doublets are due to C(8) and C(9). One doublet for both compounds has the same position (relative to progesterone) whereas the upfield doublet in 11β-hydroxyprogesterone has moved upfield (3.9 ppm). Since the 11β-OH interacts with the 8β hydrogen, the upfield shifted doublet is C(8).

The only unassigned doublet and triplet remaining are C(11) and C(12), respectively. The C(11) signal is, as expected, the most downfield sp^3 carbon for both compounds because of the attached electronegative atom.

(b) 17α-Hydroxyprogesterone. All of the carbons in rings A and B as well as C(11), C(15), C(18), C(19), and C(20) were assigned by comparison with progesterone and supported by the SFOCD spectrum. The six unassigned signals were identified by a combination of several methods. The two unassigned singlets (C(13)

Table III. Carbon-13 Chemical Shifts and Assignments for Monohydroxyprogesterone Analogs

Carbon	11α-Hydroxy	11β-Hydroxy	17α-Hydroxy	21-Hydroxy
1	33.8	34.0	35.1	35.0
2	36.9	33.4	33.5	33.4
3	198.4	197.8	198.2	197.5
4	123.5	121.5	123.1	123.0
5	171.3	172.1	171.2	170.4
6	32.8	32.4	31.9	31.8
7	31.7	31.3	30.4	31.5
8	34.2	31.0	35.1	34.8
9	58.1	55.6	53.1	52.9
10	39.5	38.8	38.2	38.0
11	67.1	66.2	20.3	20.4
12	49.4	46.8	32.3[a]	37.6
13	43.5	42.7	46.3	43.5
14	54.8	56.8	49.9	55.3
15	22.2	21.8	23.1	22.3
16	23.7	23.9	32.1[a]	23.9
17	62.3	62.9	89.2	57.4
18	14.1	15.3	14.5	13.1
19	17.9	20.3	17.0	16.7
20	208.0	208.1	210.3	209.6
21	30.8	30.9	26.6	68.6

[a] Assignments may be reversed.

(13) Roberts, *et al.* (ref 4), analyzed the cmr spectrum of 11α-hydroxyprogesterone in dioxane solution; these results cannot be compared directly to the present work which was carried out in DMSO-d_6.

Table IV. Carbon-13 Chemical Shifts and Assignments for Dihydroxyprogesterone Analogs and Cortisol

Carbon	Corti-costerone	11β,17α-Dihydroxy	Cor-texolone	Cortisol
1	34.2	34.0	35.2	34.0
2	33.5	33.4	33.4[a]	33.4
3	197.7	197.9	197.4	197.7
4	121.5	121.5	123.0	121.4
5	171.9	172.2	170.4	172.0
6	32.6	32.7	31.9[b]	32.7
7	31.5	31.4[c]	30.1	31.3
8	31.2	31.1	35.2	31.1
9	55.7	55.6	53.0	55.5
10	38.9	38.8	38.1	38.8
11	66.3	66.5	20.3	66.4
12	46.8	39.3	33.5[a]	39.0
13	43.2	45.6	47.0	46.2
14	57.1	51.4	49.9	51.5
15	22.0	23.2	23.2	23.3
16	24.2	31.9[c]	32.1[b]	32.9
17	58.2	89.1	88.4	88.3
18	15.7	16.8	14.5	16.8
19	20.5	20.4	17.0	20.4
20	209.8	209.8	211.3	211.4
21	68.6	26.5	65.9	65.8

[a], [b], [c] Carbon assignments with the same symbol (a), (b), or (c) are reversible.

and C(17)) are recognized in the SFOCD spectrum and are at 46.3 and 89.2 ppm, respectively. C(17) is predictably more downfield than C(12) due to the directly bonded hydroxyl group. C(21) is a quartet at 26.6 ppm, well separated from the C(18) and C(19) signals. The remaining doublet (49.9 ppm) must be C(14).

C(12) and C(16) have moved together (32.3 and 32.1 ppm, respectively) due to the 1,3-diaxial interaction which the 17α-OH has with H(12α), causing C(12) to move upfield (4.4 ppm relative to progesterone), and also to the electronegativity of the 17α-OH which has an inductive effect on C(16). In this instance it is not possible to make unequivocal assignments for C(12) and C(16) because of the close proximity of the two triplet signals.

(c) 21-Hydroxyprogesterone (Deoxycorticosterone). All but two signals were assigned by comparison with progesterone. The triplet (68.6 ppm) is C(21), and the doublet (57.4 ppm) is thus C(17).

Dihydroxyprogesterone Assignments (Table IV). (a) 11β,17α-Dihydroxyprogesterone. All of the assignments in this compound, except C(21), were made by comparison with cortisol. The unassigned signal at 26.5 ppm is recognized as a methyl carbon (C21)) from the SFOCD spectrum. The C(14) doublet is broad, as in previous spectra. Assignments for C(7) and C(16) may be reversed (Table II) because of the peculiar behavior of C(16), which moves upfield 1.0 ppm upon removal of the 21-hydroxyl group. Another method, such as deuteration, would be needed in order to make unambiguous assignments for C(7) and C(16).

(b) 11β,21-Dihydroxyprogesterone (Corticosterone). 11β-Hydroxyprogesterone and deoxycorticosterone are closely related structurally, and the signal positions were assigned by chemical shift comparisons.

(c) 17α-21-Dihydroxyprogesterone (Cortexolone). Assignments for carbons (low field to higher field) 20, 3, 5, 4, 17, 21, 14, 13, 10, 8 and 1 (same position), 7, 15, and 18 were made by direct comparison to cortisol and supported by the SFOCD experiment. Three carbons (9, 11, and 19) have almost the same position (±0.2 ppm) as in progesterone.

Two sets of triplets (31.9 and 32.1 ppm; 33.4 and 33.5 ppm) cannot be assigned with certainty due to the close proximity in the signal positions. The approximate position of C(2) and C(6) must be 33.4 and 31.9 ppm, respectively, by analogy to progesterone in which chemical shifts for C(2) and C(6) are 33.5 and 31.9, respectively. The signal (32.1 ppm) adjacent to 31.9 ppm is assigned to C(16). Differentiation of C(6) and C(16) was made on the basis of comparison with cortisol, but the assignments must remain tentative in this compound. The disentanglement of the remaining two triplets for C(2) and C(12) is difficult because the upfield shift of C(12) cannot be predicted exactly when the 11-OH is removed from cortisol.

Discussion

Data presented in Table V demonstrate that the hydroxyl substituent at various positions in the pregnane molecule influences the chemical shift of the α (directly attached), β, and γ carbons. Some carbons as far away as four intervening bonds are affected by the introduction of the hydroxyl group into progesterone.

Table V. Differences between Shifts of Monohydroxyprogesterone and Progesterone for α, β, and γ Carbons

	Compound	α effect[a]	β effect	γ effect
1.	11β-Hydroxyprogesterone	C(11), −45.7	C(9), −2.5; C(12), −8.9	C(8), 3.9
2.	11α-Hydroxyprogesterone	C(11), −46.6	C(9), −5.0; C(12), −11.5	C(8), 0.7
3.	17α-Hydroxyprogesterone	C(17), −26.7	C(13), −3.1; C(16), −8.3	C(12), 5.6; C(14), 5.4; C(21), 4.4
4.	21-Hydroxyprogesterone	C(21), −37.6	C(20), −1.4	C(17), 5.1

[a] Substituent shifts are in ppm relative to progesterone; a minus sign denotes a downfield shift on substitution.

To assess the effect of the 11-OH group on the progesterone conformation we compared the cmr of the 11α- and 11β-hydroxy epimers. The difference in the chemical shift for the axial and equatorial epimers in cyclohexanols[14] is usually 4–5 ppm, the axial epimer being the more upfield of the two. In the 11-hydroxyprogesterone epimers, C(11) in the 11α (axial) compound (row 1) (Table V) is upfield only 0.9 ppm more than the 11α (equatorial) isomer. This may be due to severe buttressing with the C(18) and C(19) methyls, although X-ray crystallography[2] of cortisol and related compounds did not reveal major distortions in this region of the molecule upon introduction of an 11β-OH group. It may also be due to interaction of the 11α-OH group with the 1α hydrogen (δ effect).

The magnitude of the β effect at C(9) and C(12) is

(14) J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, **92**, 1338 (1970).

dependent upon the degree of substitution on the β carbon, in agreement with the conclusions of Grutzner, *et al.*[15] We find that the C(9) carbon has a smaller downfield shift than the C(12) carbon (approximately 6.5 ppm). The 11α epimer has greater downfield shifts for both C(9) and C(12) (row 2) than the β epimer; nevertheless, the difference between C(9) and C(12) for both epimers is the same.

When the 11-hydroxyl group is moved from the equatorial (α) configuration to the axial (β) configuration, a large γ effect is observed at carbon 8 while two "pseudo" γ effects are observed at C(10) and C(13). A typical upfield shift due to 1,3-diaxial interactions in cyclohexanols is $\sim$5.0 ppm.[16] In order for the γ carbon (C(3)) in cyclohexanols to exhibit a large upfield shift of 5.0 ppm it appears that the α substituent should interact 1,3 diaxially with the hydrogen bonded to C(3). The γ carbon, C(8), has shifted upfield 3.9 ppm; thus the γ effect is smaller, but clearly present. However, C(10) and C(13) (both have methyl groups instead of hydrogen) have a very much reduced upfield shift of $\sim$0.8 ppm. The reason for the diminished upfield shift is not clear.

We observed smaller changes at various carbons even further away from the γ carbons. The δ carbons, namely C(18) and C(19), have moved downfield 1.2 and 2.4 ppm due to strong interaction with the 11β-OH in the 11β compound relative to the 11α epimer. C(1) behaves in a similar manner (2.9 ppm downfield) if the 11-hydroxyl group interacts with protons bonded to it, as in the α but not the β epimer. In comparing the α and β epimers the δ carbons have paramagnetic shift while the γ carbons have been shown to have diamagnetic shifts if increased nonbonded interaction occurs. However, Pehk[17] reported that the δ effect in biadamantane gives an *upfield* shift of 2.5 ppm.

The α and β Effects. Although the α and β effects have been discussed for the 11α and 11β epimers, similar chemical effects are observed for hydroxyls in other positions. The downfield shift of the α carbon is dependent on both the electronegativity of the oxygen and the degree of substitution involved. There is, however, no apparent trend or explanation for the observed effects (downfield shift: secondary alcohol > primary alcohol > tertiary alcohol). The β effect is again dependent on the branching of the carbon (see row 3, Table V).

The γ Effect. There is a γ effect in all of the monohydroxyprogesterone compounds (upfield shift of 3.9–5.6 ppm) with the exception of 11α-hydroxyprogesterone (row 2, Table V). The smaller effect on the γ carbons in the 11α-hydroxy compound is due to the equatorial configuration of the hydroxy group which cannot have 1,3-diaxial interactions.

Long-Range Effects. The upfield shift of C(7) (1.2 ppm) upon the introduction of a hydroxyl group at 17α on progesterone appears to be due to transmission of the buttressing effect of the OH group onto the 14α hydrogen, which in turn transmits that distortion *via* a 1,3-diaxial interaction to the 7α hydrogen. This would be a true γ effect but it is markedly diminished due to the distance effect. The α, β, and γ effects observed for the dihydroxyprogesterone derivatives and for cortisol are similar if not identical in all cases. Monohydroxy derivatives of compounds provide a clearer insight of the effect of each hydroxyl group on the pregnane structure, due to the multiplicity of hydroxyl groups in cortisol it is difficult to assess the effect of each hydroxyl group with out dealing with each hydroxyl separately as we have done.

Deuteration Effects. Deuterium labeling has been used to help identify carbons adjacent to the deuterated carbon.[18] Shifts of 0.1 ppm have been observed for the adjacent carbon in some norbornane compounds.[18] We have observed similar effects on carbons 1, 3, 5, 7, and 8 (Table VI).

Table VI. ^{13}C Chemical Shift Effects on Carbons Adjacent to a Deuterated Carbon

Compound	C(1)	C(3)	C(5)	C(7)	C(8)
Protio cortisol	34.0	197.7	172.0	31.3	31.0
Deuterio cortisol	33.9[a]	198.0[a]	172.3[a] 172.2[b]	31.4[a]	31.0

[a] Labeled at C(2) and C(6) with deuterium. [b] Labeled at C(6) and C(7) with deuterium.

Conformation of the Side Chain in Deoxycorticosterone. The conformation of the side chain is of interest on both chemical and biological grounds. X-Ray crystallography[2] indicates that the C(20) carbonyl in cortisol is oriented preferentially in the direction of the β face. Furthermore, the 21-hydroxyl substituent eclipses the 20-carbonyl group but appears to be too distant for intramolecular hydrogen bonding to occur.[2,19] Solution ir and pmr studies by Cole and Williams[20] yield the same conclusions.

Our cmr results indicate that the C(21) hydroxyl group in deoxycorticosterone is preferentially oriented on the same side as the 17α substituent. The chemical shift of the C(17) carbon for progesterone moves upfield 5.1 ppm when a hydroxyl is introduced at C(21) (deoxycorticosterone). The upfield shift is clearly due to the interaction between the 21-hydroxyl group and the C(17) hydrogen. If the 21-hydroxyl were eclipsing the carbonyl group, there should be no difference in the chemical shift of C(17), regardless of the presence of the hydroxyl groups. These results suggest that the side chain in cortisol may have the same conformation in DMSO solution. The difference between the conformation we deduce and that of others[20] is probably due to the choice of solvent, DMSO, which may form intermolecular hydrogen bonds with the various hydroxyl groups on the molecule. Since DMSO is such a highly polar solvent, the conformation we observe may be more relevant to the biological milieu than that found in a less polar solvent.

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