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MINERALOCORTICOID EFFECT AND MOLECULAR STRUCTURES IN CORTISOL AND 9α-FLUORO-SUBSTITUTED DERIVATIVES*

P. GENARD
Département de Clinique et de Pathologie Médicales, Université de Liège, Belgique

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SUMMARY

The ^{13}C-, ^{1}H- and ^{19}F magnetic resonance spectra of cortisol and of some of its 9α-fluorinated derivatives are analyzed and compared with the results obtained by X-ray diffraction.

No major conformational differences are detected after 9α-fluorination of cortisol and of prednisolone. However, modifications of the electronic structures occur in the moiety of the molecule centred on the 9α-F.

These modifications may be taken as responsible for the increase in the mineralocorticoid activity of those molecules. On the contrary the decrease in biological activity of the 9α-fluorinated derivatives methylated or hydroxylated at C_{16} is very likely dependent upon conformational and electronic changes in the D-ring and in the side chain.

INTRODUCTION

Nuclear magnetic resonance spectroscopy [1, 2] and X-ray diffraction [3] studies show that a well determined conformation of the CH_2OH-CO side chain at C_{17} is present in natural corticosteroids such as desoxycorticosterone and aldosterone which possess a high mineralocorticoid activity.

9α-Fluorocortisol and 9α-fluoroprednisolone show a mineralocorticoid activity quite similar to that of aldosterone but on the contrary, this activity is much lower when the derivative of prednisolone is either methylated or hydroxylated at C_{16} (see formulae on Fig. 1). However, the X-ray diffraction patterns show a similar molecular conformation for cortisol which has a weak mineralocorticoid effect and as well as for 9α-fluorocortisol [4]. Until now, no X-ray structure of other fluorinated compounds has been determined.

It was thus of interest to study the ^{1}H, ^{13}C and ^{19}F magnetic resonance spectra of those compounds in order to investigate possible modifications in solution of their molecular conformations electron density and polarizability of the bonds and to try and correlate them with changes in biological activity.

EXPERIMENTAL

The ^{1}H and ^{13}C spectra were obtained on a Bruker HFX-90 spectrometer equipped with Fourier Transform and operating at 90 MHz for the proton and 22.63 MHz for carbon 13. ^{13}C spectra were recorded with broad band decoupling and in off resonance conditions. ^{19}F spectra were performed on a Varian HA-100 instrument operating at 94.1 MHz. Samples were measured as 0.2–0.4 M solutions in deuteriated dimethylsulfoxyde (DMSO-d_6). Internal standards were: tetramethylsilane (TMS) for ^{1}H and ^{13}C spectra and methyltrifluoroacetate for ^{19}F spectra.

The following compounds were studied:

	Trivial name used in text
11β,17,21-Trihydroxy-4-pregnene-3,20-dione	
9α-Fluoro-11β-17,21-trihydroxy-4-pregnene-3,20-dione	
11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione	
9α-Fluoro-11β-17,21-trihydroxy-1,4-pregnadiene-3,20-dione	
9α-Fluoro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	
9α-Fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	dexamethasone
9α-Fluoro-16α-methyl-11β,21-dihydroxy-1,4-pregnadiene-3,20-dione	desoxymethasone
9α-Fluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	triamcinolone

All these compounds were obtained as a generous gift of Roussel UCLAF (Paris).

The mineralocorticoid activity of the investigated steroids has been estimated in two different ways. (1) The effect of the compound on sodium excretion in the rat using the Kagawa assay [5]; (2) The stimulatory effect of the steroid on active sodium transport evaluated by measuring the short-circuit current of the isolated urinary bladder of the toad [6].

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Table 1. ^{13}C Chemical shifts in ppm relative to TMS

C	Cortisol	Prednisolone	9α-fluorocortisol	9α-fluoroprednisolone	Dexamethasone	Betamethasone	Triamcinolone	Desoxymethasone
1	32.1	154.8	23.9	150.7	150.7	150.9	150.0	150.6
2	31.5	124.9	31.2	126.8	127.0	126.9	126.5	127.0
3	196.0	182.9	195.6	183.2	183.3	183.3	182.6	183.2
4	119.5	119.7	121.9	122.0	122.0	122.0	121.6	122.1
5	170.3	168.2	167.3	164.9	165.0	164.9	164.2	164.8
6	31.0	32.6	28.1	28.3	28.4	28.3	28.2	28.3
7	29.2	29.6	25.7	25.4	25.3	25.6	25.2	25.2
8	29.2	29.6	31.5	31.6	31.7	32.2	31.2	31.4
9	53.6	53.6	98.4	99.1	99.2	99.3	98.6	99.1
10	36.9	42.5	41.3	45.8	46.0	45.7	44.8	45.9
11	64.5	66.3	67.0	68.5	68.7	68.6	68.3	68.3
12	35.8	37.4	33.3	33.3	33.8	34.2	33.7	42.5
13	44.4	44.9	43.7	44.1	45.5	44.7	44.3	40.9
14	49.7	49.7	42.9	42.4	41.3	40.8	41.0	46.1
15	21.4	21.9	21.0	20.7	30.1	30.8	31.6	30.8
16	31.0	31.4	31.2	30.8	33.0	32.6	69.1	28.3
17	86.5	86.7	86.2	86.1	88.1	85.8	85.1	65.0
18	15.0	15.4	14.4	14.4	13.2	15.0	14.6	13.8
19	18.5	19.2	19.4	21.1	20.9	20.9	20.7	20.9
16-CH_3					14.6	17.8		19.8
20	209.6	209.3	209.3	209.1	209.0	210.2	208.7	207.7
21	63.9	64.1	63.8	63.6	64.2	65.6	64.3	66.9

The mineralocorticoid activity of prednisolone, 9a-fluoroprednisolone and desoxymethasone was estimated in our laboratory [7]. For other steroids, the data were taken from the literature [5, 6, 8].

RESULTS

Spectral assignments. ^{13}C magnetic resonance (Tables 1 and 2). The value proposed by Giannini *et al.*[9] for the ^{13}C shifts ($\delta^{13}C$) and for the coupling constants J (^{19}F-^{13}C) of cortisol and 9α-fluorocortisol are confirmed.

The spectra of prednisolone and 9α-fluoroprednisolone differ from those of cortisol and of 9α-fluorocortisol by a downfield shift of the C_1 and C_2 resonances towards the olefinic zone. Moreover C_3 is shifted to lower fields whilst C_{10} moves to higher fields (Fig. 1).

Table 2. Values of the $J(^{19}F$-$^{13}C)$ in Hertz

Steroids	$J(^{19}F - ^{13}C)$			
	C_8	C_9	C_{10}	C_{11}
9α-fluorocortisol	12.0	170.6	20.6	36.9
9α-fluoroprednisolone	21.3	174.5	26.0	38.5
Dexamethasone	19.2	174.9	22.9	38.2
Betamethasone	20.6	173.4	14.7	35.3
Triamcinolone	19.0	174.5	20.6	36.7
Desoxymethasone	19.2	176,5	23.5	36.7

The identification of the α and β methyl groups at C_{16} in dexamethasone, betamethasone and desoxymethasone is an easy matter. The presence of this CH_3 group has no marked effect on the C_{16} resonance as that of the C_{15} is shifted to lower fields.

The absence of an hydroxyl group in 17α-position in desoxymethasone and the presence of it in 16α-position in triamcinolone result respectively in an upfield shift of the C_{17} and a downfield shift of the C_{16} resonances.

In all the fluorinated compounds the resonances of the carbons in α (C_9) and in β (C_8, C_{10}, C_{11}) to the fluorine atom are shifted downfield. The carbons in γ to the fluorine bearing a β axial hydrogen (C_1, C_7, C_{12}, C_{14}) have their lines shifted upfield.

^1H magnetic resonance. Table 3 lists the chemical shifts of the 18- and 19 methyl groups for the compounds studied.

The fluorination at C_9 induces a downfield shift of about 0.1 ppm for the 19-CH_3.

The fluorine has no detectable effect on the resonance of the 18-CH_3 which is only influenced by the substitution of a methyl group or of a hydroxyl group at C_{16}.

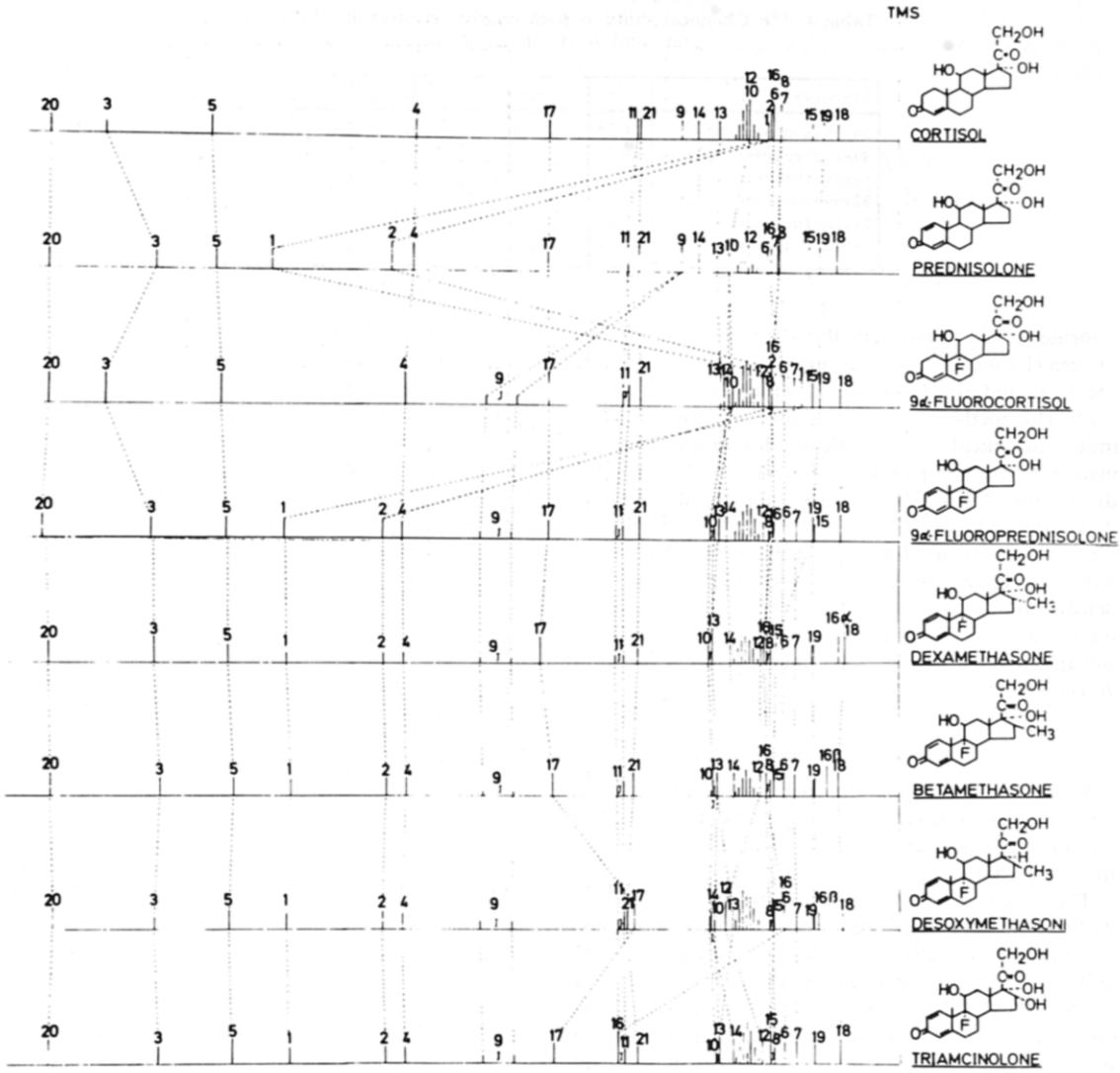

Fig. 1. ^{13}C spectra.

Table 3. Proton chemical shifts of 18-CH_3 and 19-CH_3 in ppm relative to TMS

Steroids	18-CH_3	19-CH_3
Cortisol	0.785	1.355
Prednisolone	0.775	1.390
9α-fluorocortisol	0.740	1.480
9α-fluoroprednisolone	0.760	1.475
Dexamethasone	0.860	1.475
Betamethasone	0.965	1.485
Triamcinolone	0.840	1.470
Desoxymethasone	0.815	1.480

^{19}F magnetic resonance. Table 4 shows the ^{19}F chemical shifts and the corresponding coupling constants J (^1H-^{19}F).

DISCUSSION AND CONCLUSIONS

(1) The ^{13}C magnetic resonance indicates that the fluorination at 9α leads to an expected deshielding of the carbons α and β to the fluorine. On the contrary, the carbons γ to the fluorine and bearing an α-axial hydrogen have their resonance shifted upfield. This shielding is consistent with a displacement of electrons towards the carbon atoms under the influence of the fluorine atom. Two kinds of interaction may be proposed: (a) A steric repulsion which is not very probable; indeed, the Van der Waals radius of

Table 4. ^{19}F Chemical shifts in ppm relative to methyltrifluoroacetate and $J(^1H\text{-}^{19}F)$ in Hertz

Steroids	δ	J trans(8βH-9αF)	J cis(11αH-9αF)
9α-fluorocortisol	93.3	8.5	28.7
9α-fluoroprednisolone	89.0	8.5	25.0
Dexamethasone	88.6	7.8	25.2
Betamethasone	88.4	9.7	30.0
Triamcinolone	88.7	8.2	29.6
Desoxymethasone	88.7	8.8	28.0

fluorine (1.3 Å) is not very different from that of hydrogen (1.1 Å). (b) A dipole-dipole interaction between the C–F and the C–H bonds which is more likely.

(2) The methylation or the hydroxylation at C_{16} induce modifications of conformation and of electron density at the level of the side-chain at C_{17} and of the D-ring. Those changes are substantiated by the deshielding of the $^{13}C_{15}$ itself and of the methyl protons at C_{18}. The modification in the methyl resonance indicates changes in conformation and/or of electron density at the carbonyl group at C_{20}. On the contrary, no significant influence is to be found of the substituents at C_{16} on δ ^{13}C, J ($^{19}F\text{-}^{13}C$), δ ^{19}F and $J(^1H\text{-}^{19}F)$ in the other parts of the molecule.

CONCLUSIONS

It may be concluded that within the set of fluorinated compounds studied in this work major conformational changes are unlikely to take place for the molecular moiety centred on the 9α-F.

The increase in mineralocorticoid activity of 9α-fluorocortisol and -prednisolone is most probably dependent upon electronic changes induced in the molecule by the fluorine atom: the substitution of a C_9–F for a C_9–H bond is, of course, the most important feature. Morover, the introduction of such a strong dipole modifies the polarization of the α-axial C–H bonds at C_1, C_7, C_{11} and C_{14}. Those changes are quite able to bring about some significant perturbation in the weak interactions between a steroid and its specific receptors.

The molecular site centred on C_9 is practically left unmodified by a methylation or an hydroxylation at C_{16}. Hence, the weaker activity of these compounds may be confidently ascribed to conformational and electronic modifications both in the D-ring and in the side chain at C_{17}.

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