# A Comparison of the Molecular Structures of Six Corticosteroids

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**Abstract:** A comparison of the molecular conformations of cortisol, 6α-fluorocortisol, 9α-fluorocortisol, and 6α-methylprednisolone, as determined by X-ray analysis, is relevant for purposes of relating conformational differences and biological activity. In 9α-fluorocortisol, the A ring is bent underneath the plane of the molecule to a much greater extent than it is in cortisol, and this bend, which may result from a close nonbonded contact between the fluorine atom and the axial substituent on C(1), resembles the conformational change occasioned by 1–2 dehydrogenation. A correlation was noted between the degree of bowing toward the α face and the variation in anti-inflammatory activity in this series of corticosteroids. In all six corticoids for which the 17β side chains were compared, atoms O(20) and O(21) are cis coplanar, and O(20) is oriented over the D ring as predicted by Wellman and Djerassi from solution spectral analysis. Intramolecular hydrogen bonding to the 17α-hydroxyl group is not observed, and intramolecular hydrogen bonding within the side chain, if any, is weak and of highly distorted geometry. Although the 17β side-chain conformation seems nearly invariant, a slight variation is observed in 6α-fluorocortisol which is the only structure in which O(20) is involved in a hydrogen bond.

The adrenal cortex of man and the higher animals produces a number of steroid hormones which have a wide variety of physiological functions. The three major types of biological responses elicited by the adrenocortical hormones are (1) alterations in carbohydrate metabolism which are monitored by changes in liver glycogen deposition, (2) antiinflammatory activity, and (3) maintenance of proper salt and water balance through effects on the rate of $Na^+$ excretion from the kidneys. The natural glucorticoid hormones cortisol (Figure 1) and cortisone influence all three activities but have comparatively weak effects on electrolyte metabolism. Cortisol and cortisone alleviate the symptoms of rheumatoid arthritis on account of their antiinflammatory activities, but therapeutic doses produce deleterious side effects which result primarily from changes in electrolyte metabolism.

In the 1950's, a largely empirical search was made for drugs which had the high antiinflammatory potency of cortisol, but which did not alter electrolyte metabolism. A 9α-fluorine atom increases antiinflammatory activity by a factor of 7–10,[2] but it also has the undesirable effect of substantially increasing sodium retention. Substitution of the other halogens for the 9α-hydrogen also causes the activities to change, and antiinflammatory activity decreases with increasing size of the halogen. 6α-Fluorination causes a much smaller increase in antiinflammatory activity than does 9α-fluorination. Dehydrogenation of the 1–2 bond, but not the 6–7 bond, increases antiinflammatory activity without the undesirable increase in sodium retention which accompanies 9α-fluorination. The effects of 1-dehydrogenation and 9α-fluorination are cumulative, and while the doubly modified steroid has very favorable antiinflammatory properties, it also causes sodium retention. This sodium retention can be eliminated by addition of either a 16α-hydroxyl or methyl group.

The changes in activity accompanying 9α-halogenation were first thought to result from electronic effects. However, a comparison of the molecular geometry of the 9α-fluorocortisol molecule, as determined by X-ray analysis, with the structures of cortisol, 6α-fluorocortisol, and 6α-methylprednisolone (6α-methyl-1-dehydrocortisol) suggests that the increased activity of the 9α-fluoro derivative may result from an unexpected change in the A-ring conformation.

## Experimental Section

In this paper, the salient features of the molecular structures of six corticosteroids are compared. These structures have all been determined by X-ray analysis from three-dimensional diffraction data, and the nonhydrogen atoms have been refined with anisotropic thermal parameters. In all cases, most or all of the hydrogen atoms have been found by Fourier difference syntheses. The six structures which are compared are cortisol ($R < 6\%$),[3] 6α-fluorocortisol ($R = 7.3\%$),[4] 9α-fluorocortisol ($R = 5.6\%$,[5] $R = 4.6\%$[6]), 6α-methylprednisolone ($R = 3.6\%$),[7a] cortisone ($R = 5.8\%$),[7b] and 4-chlorocortisone ($R = 7.7\%$).[8] The crystallographic $R$ values ($R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$), which measure the agreement of the observed and calculated structure factors for the atomic positions, show that all of these structure determinations are good and that the standard deviations of the atomic coordinates are of the order of 0.005 Å for carbon–carbon bond lengths and 0.5° for nonhydrogen atoms. The crystal structures of other corticosteroids are known,[9,10] but because of the presence of bulky substituents on the side chains, they were not considered suitable for the present comparison.

The structure of 9α-fluorocortisol was solved by a combination of direct method techniques and was facilitated by a consideration of the cosine seminvariants, $\cos(\phi_1 + \phi_2)$.[11] The final $R$ value for the 1627 reflections having observed intensities more than twice their

(1) (a) The Medical Foundation of Buffalo; (b) University of California.

(2) J. Fried, "Biological Activities of Steroids in Relation to Cancer," G. Pincus and E. P. Vollmer, Ed., Academic Press, New York, N. Y., 1961, pp 9–23.

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(6) L. Dupont, O. Dideberg, and H. Compsteyn, *Cryst. Struct. Commun.*, **1**, 177 (1972).

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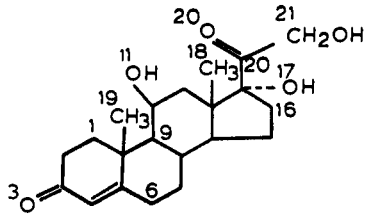

Figure 1. Cortisol.

calculated standard deviations was 5.6%, and the $R$ value for all data was 6.2%. Tabulations of the crystal data, bond lengths, valency angles, the observed and calculated structure factor amplitudes, and the atomic positional and thermal parameters from this determination are available from the authors upon request. The steroid geometry observed in this determination is in good agreement with that observed by Dupont, Dideberg, and Compsteyn.[6] Two bond lengths, C(5)–C(10) and C(21)–O(21), differ by 0.021 and 0.018 Å, respectively, and each of the valency angles C(2)–C(3)–O(3), C(4)–C(5)–C(6), C(10)–C(5)–C(6), and C(12)–C(13)–C(18) differs by 1.2°. The two determinations agree in all other bond lengths and angles to within twice the estimated standard deviations.

## Geometry near Fluorinated Substituents

In order to investigate the possibility that electronic effects cause the differences in biological activity between cortisol and 9α-fluorocortisol, the distances and angles in the region of C(9) in these structures were compared to each other and to the distances and angles in 6α-fluorocortisol as shown in Table I. The presence

Table I. Interatomic Distances (Å) and Valency Angles (deg) in Regions near Fluorine Atoms

| | Cortisol | 6α-Fluorocortisol | 9α-Fluorocortisol |
|---|---|---|---|
| C(6)–C(5) | 1.498 | 1.468 | 1.498 (1.496)[a] |
| C(6)–C(7) | 1.519 | 1.518 | 1.540 (1.541) |
| C(6)–F(6) | | 1.388 | |
| C(9)–C(8) | 1.546 | 1.537 | 1.529 (1.538) |
| C(9)–C(10) | 1.573 | 1.577 | 1.573 (1.565) |
| C(9)–C(11) | 1.541 | 1.534 | 1.539 (1.544) |
| C(9)–F(9) | | | 1.436 (1.439) |
| C(5)–C(6)–C(7) | 112.9 | 112.9 | 113.5 (112.8) |
| C(5)–C(6)–F(6) | | 110.3 | |
| C(7)–C(6)–F(6) | | 109.1 | |
| C(8)–C(9)–C(10) | 112.7 | 114.9 | 114.0 (13.9) |
| C(8)–C(9)–C(11) | 115.3 | 113.7 | 114.5 (114.2) |
| C(10)–C(9)–C(11) | 113.9 | 113.9 | 114.6 (114.9) |
| C(8)–C(9)–F(9) | | | 105.0 (105.2) |
| C(10)–C(9)–F(9) | | | 103.6 (104.0) |
| C(11)–C(9)–F(9) | | | 103.2 (102.8) |

[a] The values in parentheses are from ref 6.

of the 9α-fluorine atom has not caused any significant differences in any of the carbon–carbon bonds involving C(9), nor has any change occurred in the valency angles centered about C(9), and if the presence of the 9α-fluorine has induced any electronic changes in the rest of the molecule, these changes are smaller than the estimated standard deviations in the values of the distances and angles. Despite the absence of large distortions of bond lengths and angles, CNDO/2 molecular orbital calculations based upon the observed atomic coordinates indicate that two types of electronic effects are present: local direct inductive effects and alterations in charge distribution accompanying conformational changes.[12]

(12) P. A. Kollman, D. Giannini, W. L. Duax, S. Rothenberg, and M. E. Wolff, *J. Amer. Chem. Soc.*, **95**, 2869 (1973).

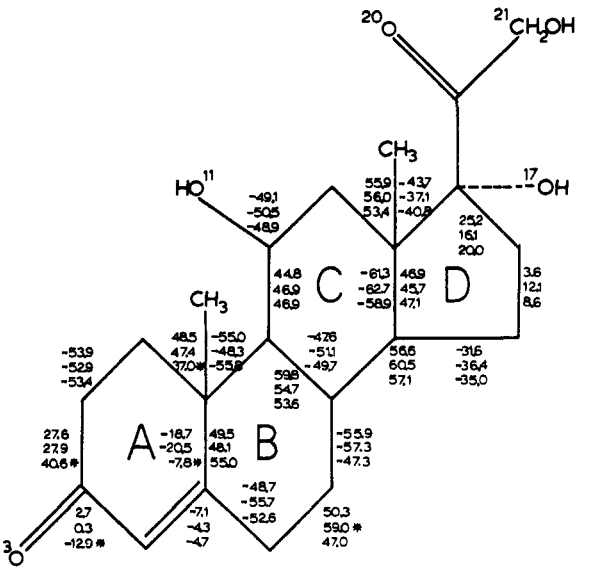

Figure 2. Torsional angles in the rings of cortisol and its fluorinated derivatives. For each angle, the value for cortisol is given first, then the value for 6α-fluorocortisol, and finally, the value for 9α-fluorocortisol. In each case, the torsional angle is centered about the bond on which the figures are given, and the other two atoms are in the same ring. The asterisk denotes that the angle for one of the structures differs from the values for the other structure by *ca.* 10°.

Some differences are observed, however, when the geometry about F(9) in 9α-fluorocortisol is compared with the geometry about F(6) in 6α-fluorocortisol. The difference of 0.03 Å between the C(6)–C(5) distance in 6α-fluorocortisol and either cortisol or 9α-fluorocortisol is greater by a factor of 4–5 than the standard deviations in bond lengths in any of these structures. Also, the C(6)–F(6) distance of 1.388 Å in 6α-fluorocortisol is almost equal to the average value given in "International Tables for X-Ray Crystallography"[13] for carbon–fluorine bond lengths and is 0.05 Å shorter than the C(9)–F(9) bond length of 1.436 Å in 9α-fluorocortisol. In addition, the valency angles of 105.0, 103.6, and 103.2° which involve F(9) are less than the tetrahedral value, whereas those involving F(6) in 6α-fluorocortisol are not. The larger value of the carbon–fluorine distance seen in 9α-fluorocortisol agrees well with the values of 1.431 and 1.421 Å observed for the C(6)–F(6) distances in 6β-fluoro-17β-acetoxy-6α-methyl-9β,10α-androst-4-en-3-one and 6α-fluoro-17β-acetoxy-6β-methyl-9β,10α-androst-4-en-3-one.[14] Both of these retrosteroids differ from 6α-fluorocortisol in the C(6) region in that there is a methyl carbon bonded to C(6) instead of a hydrogen atom. The androstane derivatives also have valency angles involving fluorine which are less than the tetrahedral value. Thus, similar geometry is seen in the three cases where the carbon bonded to the fluorine atom is also bonded to three other carbon atoms. However, it is doubtful whether the observed change in the C(5)–C(6) distance in 6α-fluorocortisol is biologically significant, especially in view of the fact that 6α-fluorocortisol has activity intermediate between cortisol and 9α-fluorocortisol.

(13) "International Tables for X-Ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1965.

(14) P. B. Braun, J. Hornstra, and J. I. Leenhouts, *Philips Res. Rep.*, 24, 450 (1969).

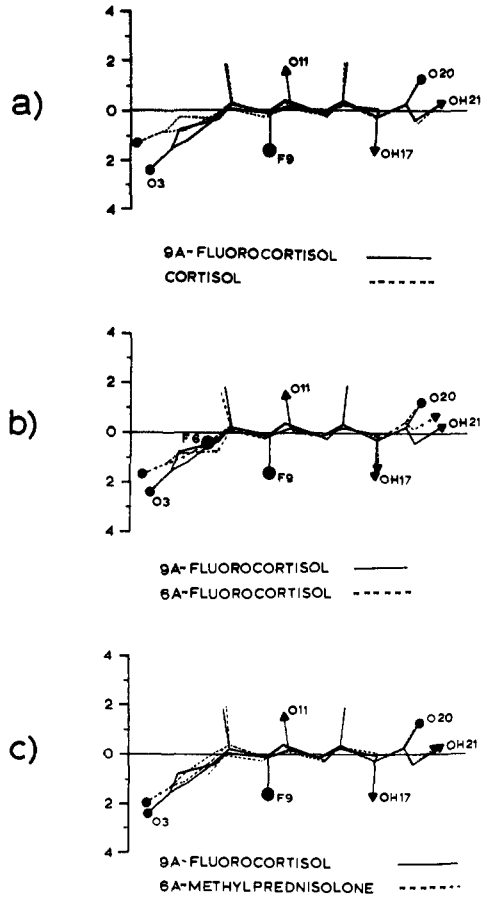


Figure 3. Projections parallel to the least-squares plane passed through atoms C(5) to C(17) inclusive. The molecules of (a) cortisol, (b) 6$\alpha$-fluorocortisol, and (c) 6$\alpha$-methylprednisolone are successively imposed on the 9$\alpha$-fluorocortisol molecule.

## A-Ring Conformation

Since comparison of the interatomic distances and valency angles for cortisol and its fluorinated substituents did not reveal any differences which might be correlated with differences in any of their biological activities, the torsional angles for these structures were next examined, and many of these angles are given in Figure 2. Those cases where the angle for one of the three structures differs from the value for the other two structures by about 10° or more are indicated by asterisks. Of the five angles in this group, all but one is an angle in the A ring of 9$\alpha$-fluorocortisol, so it is apparent that the A ring conformation in this structure is quite different from the conformation seen in the other two structures.

The way in which the A ring of 9$\alpha$-fluorocortisol differs from the analogous region in the other cortisols can be seen more clearly if the molecules are viewed in a projection parallel to the least-squares plane passed through atoms C(5) to C(17) inclusive. In Figure 3, 9$\alpha$-fluorocortisol is seen in this projection with cortisol, 6$\alpha$-fluorocortisol, and 6$\alpha$-methylprednisolone successively imposed upon it. It is immediately apparent that the A ring in 9$\alpha$-fluorocortisol is bent underneath the molecule to a much greater extent than it is in cortisol. The A-ring orientation in 6$\alpha$-fluorocortisol and 6$\alpha$-methylprednisolone is intermediate between the A ring conformations of cortisol and 9$\alpha$-fluorocortisol, and the degree of bend in 6$\alpha$-methylprednisolone bears

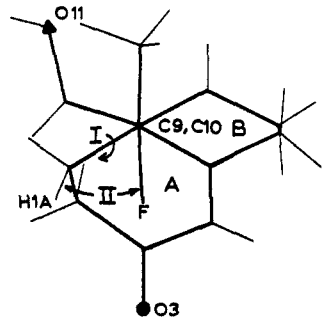


Figure 4. Projection of the A and B rings of the 9$\alpha$-fluorocortisol molecule down the C(10)–C(9) bond.

the closest resemblance to that seen in 9$\alpha$-fluorocortisol. From inspection of a Dreiding model of 6$\alpha$-methylprednisolone, it can be seen that the presence of the 1–2 double bond will force the A ring to adopt a conformation similar to what is actually observed in the crystal structure. However, the reason for the sharp bowing of the A ring toward the $\alpha$ side in 9$\alpha$-fluorocortisol is not so obvious. As shown in Figure 4 where the A and B rings of the 9$\alpha$-fluorocortisol molecule are viewed in projection down the C(10)–C(9) bond, there is a close nonbonded contact between the axial hydrogen on C(1) and the 9$\alpha$ substituent, whether this substituent is fluorine or hydrogen. This close contact, which is labeled II on the figure, may be relieved to some extent by a rotation about the C(1)–C(10) bond which also causes a change in the torsion angle C(2)–C(1)–C(10)–C(9) which is labeled I. In cortisol and 6$\alpha$-fluorocortisol this torsion angle has a value of about 165°, but in 9$\alpha$-fluorocortisol, the rotation has changed the angle to 155°. The theoretical F(9) position in cortisol was calculated by extending the C(9)–H(9) bond, and the distance from this theoretical position to the axial H(1) was found to be 2.25 Å which is 0.1 Å less than the distance actually seen in 9$\alpha$-fluorocortisol. The angle for 6$\alpha$-methylprednisolone is not really comparable to the other values because the 1–2 double bond changes the geometry in this region so greatly. It is apparent, however, that the 9$\alpha$-fluoro substituent has changed the cortisol geometry such that it more closely resembles that of prednisolone. This change in the C(1) region forces compensating alterations in the rest of the A ring so that the overall result is the greater deviation of the A-ring atoms from the plane formed by the remainder of the steroid nucleus which was seen in Figure 3 and which may be expressed quantitatively in terms of the distance of O(3) to the plane through C(5)–C(17) as shown in Table II. Thus, these cortisol structures form the same series when ordered either

Table II. Some Quantitative Measures of A-Ring Conformational Differences in Cortisol and Its Derivatives[a]

| | I | II | III |
|---|---|---|---|
| Cortisol | 165.5 | 2.23[b] | 1.32 |
| 6$\alpha$-Fluorocortisol | 164.3 | 2.08 | 1.77 |
| 9$\alpha$-Fluorocortisol | 154.6 | 2.34 | 2.43 |
| 6$\alpha$-Methylprednisolone | 122.1 | 3.16 | 1.95 |

[a] (I) Torsional angle (deg) C(2)–C(1)–C(10)–C(9); (II) H(9)–axial H(1) or F(9)–axial H(1) distance (Å); (III) distance from O(3) to C(5)–C(17) plane (Å). [b] Cortisol axial H(1) to theoretical F(9) distance = 2.25 Å.

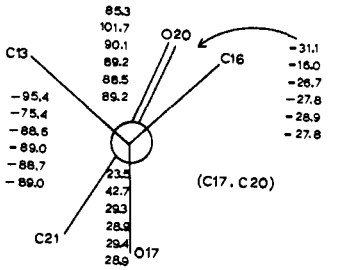


Figure 5. Geometry of the C(17) side chain. The torsional angles for the angles seen in the Newman projection down the C(17)–C(20) bond are given in the order (a) cortisol, (b) 6α-fluorocortisol, (c) 9α-fluorocortisol, (d) 6α-methylprednisolone, (e) cortisone, and (f) 4-chlorocortisone.

according to A-ring conformation or antiinflammatory activity, and this observation leads to the speculation that a cause and effect relationship may exist.

## Side-Chain Conformation and Hydrogen Bonds

The torsion angles and least-squares projections which have been examined have shown that the only appreciable differences in the nucleus of 9α-fluorocortisol and nuclei of the other cortisol structures were in the A-ring region. The geometry of the side chain at C(17) in the cortisols and the two cortisone structures which have been analyzed is compared in Figure 5 and Table III. The side-chain conformation, which is seen

Table III. Torsional Angles O(20)–C(20)–C(21)–O(21) in the Corticosteroid Structures

| Structure | O(20)–C(20)–C(21)–O(21), deg |
|---|---|
| Cortisol | −10.6 |
| 6α-Fluorocortisol | 6.7 |
| 9α-Fluorocortisol | −2.1 |
| 6α-Methylprednisolone | −8.8 |
| Cortisone | −0.6 |
| 4-Chlorocortisone | −8.8 |

in Figure 5 in Newman projection down the C(17)–C(20) bond, agrees qualitatively in all cases with the conformation assigned by Wellman and Djerassi[15] on the basis of optical rotatory dispersion and circular dichroism measurements. The exact orientation of the side chain in 6α-fluorocortisol is somewhat different from what is observed in all the other structures, and the torsion angles for this structure differ by about 10°. This conformational difference almost certainly arises because O(20) serves as an acceptor in a hydrogen bond only in the 6α-fluorocortisol structure. The change in side-chain orientation in this structure also induces the D ring to adopt a half-chair conformation although the D ring has a β-envelope conformation in all other cases. The two oxygen–oxygen hydrogen bonds in 9α-fluorocortisol, O(17)–O(21) and O(21)–O(3), are 2.78 and 2.79 Å, respectively, and the hydrogen is located nearly between the donor and acceptor as shown by the donor–H···acceptor angles of 160° or greater. The O(11)–F(9) hydrogen bond is longer (3.01 Å), and the donor–H···acceptor angle is 133°. O(3), which always participates in at least one hydrogen bond, is the favored hydrogen acceptor in these structures, and this is consistent with the exposed location of this atom. The approach to O(20) is hindered by the C(18) methyl group and by the hydrogen on O(21), and in the one instance (6α-fluorocortisol) where it does serve as a hydrogen acceptor, the hydrogen bond is relatively long (2.94 Å) and strained (donor–H···acceptor angle = 121°).

(15) K. M. Wellman and C. Djerassi, *J. Amer. Chem. Soc.*, 87, 60 (1965).

## Summary

When the molecular geometries of cortisol, 6α-fluorocortisol, and 9α-fluorocortisol are compared, the minor variations in bond lengths and torsional angles that are observed suggest that electronic effects introduced by fluorine substitution are primarily local and do not play a significant role in altering steroid conformation in this series.

CNDO/2 molecular orbital calculations based upon the observed atomic coordinates indicate that in addition to local direct inductive effects, significant alterations in charge distributions accompany molecular conformational changes.[12] When the torsional angles and overall conformation of these corticoids and 6α-methylprednisolone are compared, a gradual bowing toward the α side is observed that parallels the variation in antiinflammatory activity observed within the series. The 17β side chain of these steroids as well as cortisone and 4-chlorocortisone have nearly identical conformations with atoms O(20) and O(21) oriented cis-coplanar, O(20) oriented over the D ring, and no evidence of intramolecular hydrogen bonding. The 17β side chain most at variance with the others is that of 6α-fluorocortisol which is the only one in which O(20) is involved in a hydrogen bond.

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