Quantitation of Long-Range Effects in Steroids by Molecular Orbital Calculations[1a]

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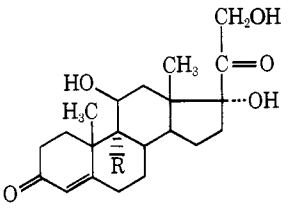
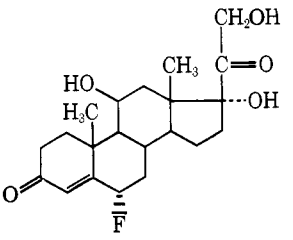
Abstract: The calculation of electron densities in portions of cortisol (**1**), 6α-fluorocortisol (9), and 9α-fluorocortisol (**2**) using X-ray structures and the CNDO/2 method is described. It is shown that the long-range influence of a substituent group on another portion of the steroid has two components: conformational steric transmission (CST) and conformational electronic transmission (CET). The implications of this for biological activity of **1, 2,** and 9 are discussed. The effects of electronegative and electropositive substitution on the H-bonding ability of ethanol (both as proton donor and acceptor) are examined by the CNDO/2 molecular orbital method, and the results are related to the steroid hydroxyl electron densities.

A number of examples of long-range effects of substituent groups on chemical processes in steroids and related compounds exist in the literature. Such effects have been rationalized by invoking one or a combination of three possible mechanisms: (1) conformational transmission, (2) inductive effects, and (3) "through-space" electrostatic effects. For example, the marked alteration in the rate of condensation of steroidal or triterpenoid 3-ketones by structural modifications in other rings,[2] which cannot be explained simply on the basis of electrostatic or bond induction effects, has been rationalized in terms of distortion of bond angles caused by the introduction of unsaturated linkages (conformational transmission). However, there are other examples of the passing on of apparent inductive effects over long distances,[3] such as the influence of substituents at C-17 on the rate of bromination of Δ^5 steroids or the changes in the rate of solvolysis of 17β-tosylates of steroids having varying substituents in rings A and B,[4] which cannot be understood in these terms. Although some authors ascribe such long-range phenomena to simple inductive communication along σ bonds using multiple pathways,[5] others[6] consider such a passage across more than one or two σ bonds unlikely, and that in fact a field effect is operative. The existence of such field effects in the transmission of inductive phenomena between C-4 and C-17 has been claimed.[7]

The importance of the true nature of these long-range effects in steroids is not limited only to their influence on chemical reactivity. Since relatively small changes in steroid structure lead to quantitative or qualitative changes in the hormonal activity of steroids or of their medicinal analogs, it seems clear that the means by which substituents transmit their effect to remote positions is of fundamental importance in understanding both the chemistry and the biology of steroids and related natural products. The particular problem which interested us in this connection is the change in glucocorticoid activity associated with fluorination at the 6α (9) and the 9α (**2**) positions of cortisol (**1**).[8] Both of



1, R = H
2, R = F
3, R = Cl
4, R = Br
5, R = I
6, R = OH
7, R = OCH_3
8, R = Me



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the fluorinated compounds show about a tenfold increase in glucocorticoid activity relative to the parent compound. The higher 9α-halogens elicit lower activity (**3**, 4.7; **4**, 0.3; **5**, 0.1), whereas the 9α-hydroxy derivative (**6**) is weakly active (0.2) and the 9α-methoxy (**7**) and 9α-methyl (**8**) congeners are inactive. The lack of activity in **7** was confirmed by resynthesis and testing.[9]

Since the discovery of activity-enhancing groups in steroids, efforts have been made to quantitate the effects of these groups[10] and to explain their action. In doing this, it is necessary to consider the effect of such groups

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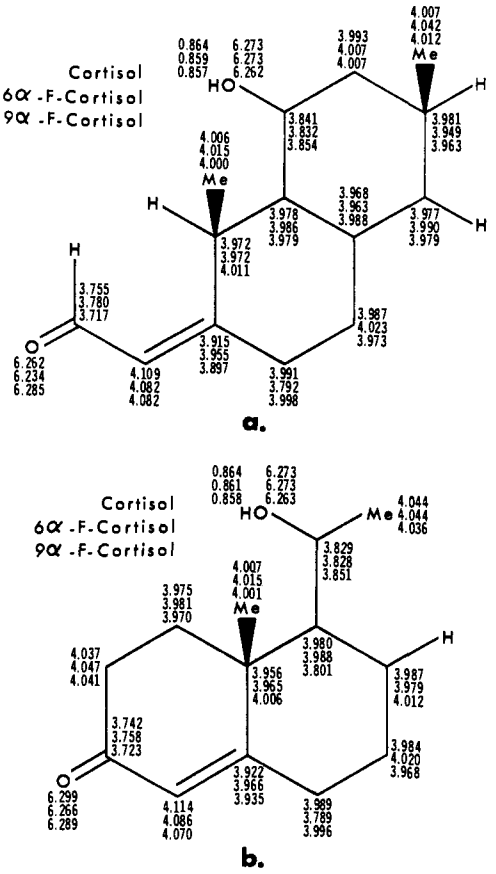

Figure 1. (a) Electron densities of carbon, oxygen, and alcoholic hydrogen atoms in cortisol, 6α-fluorocortisol, and 9α-fluorocortisol. This structure has closed B and C rings. (b) Same as (a) with closed A ring. The actual X-ray coordinates of all three cortisol derivatives were used.

on a number of part-processes in drug action,[11] *viz.*, drug distribution and drug metabolism, drug receptor affinity, and drug intrinsic activity. We may ask, therefore, by what means the 9α substituent could influence these processes. There is already some evidence which has been adduced regarding this question. Fried[10] pointed out that biological activity in 9α-halogenated cortisol derivatives varies directly with the electronegativity of the halogen, whereas Wolff and Hansch[9] correlated the steric, inductive, and hydrophobic bonding properties of the 9α group with the biological response. Moreover, we have obtained evidence[12] that at least some of the activity-modifying effect of these groups is by alteration in the strength of binding to cytosol receptors.

Fried[10] suggested that the enhancing influence of electronegative 9α groups is due to their effect on the 11β-hydroxy group and there is considerable support for this.[8] But as long-range phenomena are demonstrably present in steroids, an alteration at C-9 could be transmitted throughout the whole of the steroid framework.

In the following, we make an attempt to understand long-range effects in steroids by carrying out electronic structure calculations on substituted glucocorticoids using the recently solved X-ray structures for these compounds. These computations are made on the basis of the crystal structure of the hormone or analog itself, rather than on a brominated product or complexed derivative[13] or by direct calculation of the molecular energy using standard bond lengths and angles[14] since, as will be shown, geometrical distortions are very important in understanding electron density changes.

Computational Details

The coordinates for the carbon, oxygen, and fluorine atoms were taken from the X-ray data.[15,16] All C—H bonds were taken as 1.11 Å; all O—H bonds were made 0.97 Å. In reported X-ray structures, C—H distances varied considerably (from 0.9 to 1.2 Å) reflecting the uncertainty in hydrogen determination by X-ray methods. When only a fragment of the steroid was considered in the calculation, the new C—H bond was pointed in the same direction as the broken C—C bond it replaced with R(C—H) = 1.11 Å.

The molecular orbital calculations were carried out using the CNDO/2[17] method (standard parameterization). One of the strengths of this method is its ability to provide reasonable charge distribution in molecules. Since it is changes in charge distribution with which we are concerned, this method should give qualitatively useful information.

In our calculations on cortisol and its 6α-F and 9α-F derivatives, we have carried out the SCF calculations on "hypothetical" 6α-F and 9α-F derivatives by substituting the fluorine at the 6 and 9 positions, using the cortisol coordinates and scaling the C—F bond to 1.40 Å (Figure 3) *and* by using the actual X-ray coordinates for 6α-fluoro- and 9α-fluorocortisol. This procedure allows us to separarate the fluorine-inductive effect (which one determines using cortisol coordinates, substituting the fluorine to construct the "hypothetical" fluoro derivative and comparing the electron density of the substituted and unsubstituted compounds) from the effect of steric changes on the electron density. By comparing the results of the calculation with fluorine substituted into cortisol coordinates with the true F-substituted X-ray data, one obtains the steric perturbation effect on electron density.

Results and Discussion

We examined three steroids: cortisol, 9α-fluorocortisol, and 6α-fluorocortisol. To determine the magnitude of the three mechanisms for long-range transmission of substituent effects already mentioned (conformational transmission, inductive effects, and "through space" or "field" electrostatic effects), we carried out CNDO/2 molecular orbital calculations on fragments of the A, B, and C rings of these compounds using X-ray structural data. The electron distribution from these calculations is presented in Figure 1, and the electron density difference between 6α-fluoro- and 9α-fluorocortisol and the parent compound is presented in Figure 2.

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(13) H. Repmann, *Theor. Chim. Acta*, **17**, 396 (1970).
(14) J. Caillet and B. Pullman, *ibid.*, **17**, 377 (1970).
(15) The coordinates for 6α-fluoro- and 9α-fluorocortisol are given by C. M. Weeks, W. L. Duax, and M. E. Wolff, *J. Amer. Chem. Soc.*, **95**, 2865 (1973).
(16) The coordinates for cortisol were kindly supplied by Dr. Paul Roberts, Department of Chemistry, Cambridge University, to whom we express best thanks.
(17) J. A. Pople, D. P. Santry, and G. A. Segal, *J. Chem. Phys.*, **43**, S129, S136 (1965); **44**, 3289 (1966).

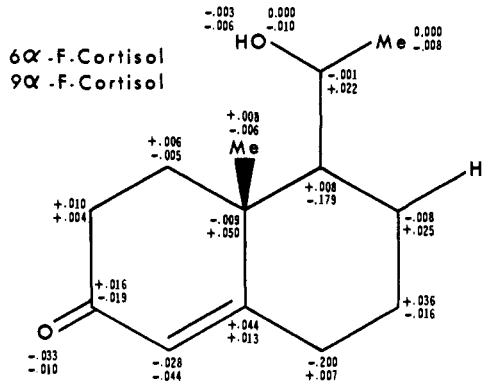

Figure 2. Electron density differences between cortisol and its fluoro derivatives. A negative density indicates loss of electron density relative to cortisol. The actual X-ray coordinates of all three cortisol derivatives were used.

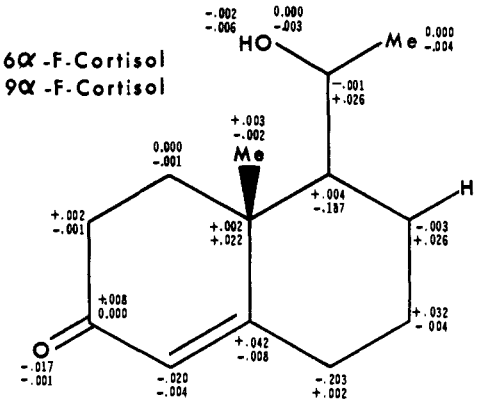

Figure 3. Electron density differences due to direct inductive effects of fluorine substituted for hydrogen in cortisol. The X-ray structure of cortisol was used in the calculations, with fluorine substituted in the 6α and 9α positions.

(a) **Inductive Effects.** What is the direct inductive effect of these substituents? We have examined this question by using the cortisol coordinates and directly substituting fluorine at the 6α and 9α positions. The electron density differences for these substitutions (relative to cortisol) are presented in Figure 3. We have also carried out calculations on steroids with a hydrogen substituted at the 9α and 6α positions using, respectively, the coordinates for 6α- and 9α-F-substituted compounds (Figure 4). Comparison of Figures 2 and 3 supports the premise that direct inductive effects are less important than geometry changes in causing alteration of electron density at carbons in ring A. In Figure 4, we have examined the direct inductive effect of substituting a hydrogen in the 6α- and 9α-F positions using the X-ray data from the fluoro-substituted compounds. Direct inductive effects die out quickly as one moves away from the position of substitution and are most important at the carbon to which the fluorine is attached.

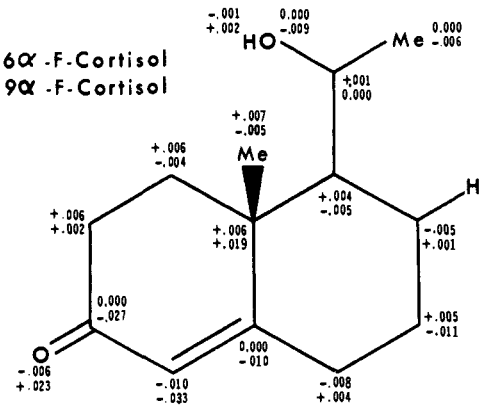

Figure 4. Calculated electron density differences between cortisol using its own X-ray coordinates and for fluorine 6α-fluorocortisol and 9α-fluorocortisol coordinates (in each case substituting a hydrogen at the appropriate position).

(b) **Field Effects.** Next, we turned to the question of the magnitude of "field" (through space) effects as discussed in the introduction. We examined this possibility using ethylene as our model system (in the xy plane) and methane and methyl fluoride. We tried to "mimic" the field effect of 17α- and 17β-electronegative group substitution on the double bond in Δ^5 steroids by placing a methane or a methyl fluoride (fluorine pointing away from ethylene) along the x axis or parallel to the π bond (in the z direction). At the distance considered (5 Å) which is somewhat less than the C-17 → C-5 distance in the steroids, there is no significant (≤ 0.001 electron) density shift in the ethylene or energy difference between the two orientations.

What do these calculations have to say in a general way about "long-range" substituent phenomena in steroids? First, direct field effects from nonionized groups, especially 17-position effects on Δ^5 steroids, will probably have little effect on reaction rates or other phenomena controlled by electronic factors. Secondly, inductive forces fall off rapidly when transmitted through a few aliphatic bonds, although they do play some role in transmission through unsaturated systems (*e.g.*, $C{=}O^3{-}C^4{=}C^5$).

(c) **Conformational Transmission.** Having discussed both inductive and field effects, we are left with "conformational transmission" as the most likely responsible mechanism for long-range substituent influence. The conventional conceptual use of "conformational transmission" has considered only stereochemical changes; here we show that the accompanying changes in charge densities are important and often significantly outweigh direct inductive electronic effects. In the light of these findings, we find it convenient to divide "conformational transmission" (CT) into two parts: conformational steric transmission (CST) and conformational electronic transmission (CET). By CST, we mean essentially the phenomena previously attributed solely to CT, *viz.*, the acceleration or retardation of reaction velocities by long-range conformational factors affecting the geometry of starting material, product, or transition state. By CET, we mean long-range electronic changes other than inductive or field effects caused by these conformational changes.

For example, in comparing the electron densities in the A ring of 9α-fluorocortisol with those in cortisol using the X-ray coordinates for both (Figure 2), the differences at C_5, C_4, C_3, and O_3 are +0.013, −0.044, −0.019, and −0.010, respectively (negative means more electrons in cortisol than in 9α-fluorocortisol). By contrast, comparing the electron-density differences for 9α-F substitution using the cortisol coordinates for both (Figure 3), the differences for the same positions are −0.008, −0.004, −0.000, and −0.001. In

Table I. Results of H-Bonding Study of XCH_2CHYOH as Proton Donor and Acceptor

ΔE(pd),[a] kcal/mol	ΔE(pa),[b] kcal/mol	ρ(H)[c]	ρ(O)[d]	Substituent[e]
8.34	5.52	0.875	6.242	(X = H, Y = H)
9.28	4.95	0.864	6.237	9α-F (X = F, Y = H)
9.34	4.33	0.864	6.240	9α-CF_3 (X = CF_3, Y = H)
8.72	5.33	0.870	6.242	9α-OH (X = OH, Y = H)
9.47	4.89	0.862	6.236	9α-OCF_3 (X = OCF_3, Y = H)
8.21	5.20	0.877	6.245	9α-Me (X = CH_3, Y = H)
10.22	4.26	0.855	6.229	11α-CF_3 (X = H, Y = CF_3)
8.21	6.77	0.877	6.252	11α-Me (X = H, Y = CH_3)
8.68	5.30	0.870	6.240	9α-OCH_3 (X = OCH_3, Y = H)

[a] H bond of substituted ethanol as *proton donor* to water. [b] H bond of substituted ethanol as *proton acceptor* from water. [c] Electron density at alcoholic hydrogen in substituted ethanol. [d] Electron density at alcoholic oxygen in substituted ethanol. [e] R(OH) = 1.03 Å was used in these model calculations.

making the same comparison for 6α-F substitution (Figure 3), the differences at C_5 and C_4 are dominated by direct inductive effects, but the inductive effect would predict the electron density at C_3 and O_3 to be +0.008 and −0.017, and the values found using the X-ray data are −0.016 and −0.033. Note that in some cases, *e.g.*, C-5, the sign of the electron density change is opposite to that predicted by a direct inductive effect. If the CET and inductive effects of substitutions such as 6α-F and 9α-F were independent effects, addition of the charge density differences of Figure 3 (inductive) and Figure 4 (CET) would yield the differences in Figure 2. As one can see from comparison of the figures, the two electron density effects (inductive and CET) are only roughly additive.

To investigate the direct role of 9α and 11α substitution on the 11β-hydroxy group, we have carried out CNDO/2 calculations examining the hydrogen bonding of ethanol with water (a 9α substitution means replacement of the hydrogen *trans* to the hydroxyl, whereas an 11α substitution involves a geminal H substitution). The results of this hydrogen-bond study are presented in Table I. Both the proton donor and proton acceptor abilities of the substituted ethanol were examined as a function of the original electron density of the ethanol oxygen and its hydrogen. Although the absolute values of the H-bond energy are exaggerated by 50–100%, one should be able to deduce the order of magnitude of the energy change due to 9α and 11α substitution. All the minimum energy values occurred near (R(O···O) = 2.55 Å) and the values at this distance are quoted in the table. No clear correlation of ethanol proton acceptor ability with *oxygen* electron density is obvious from the data, but the positive character of the proton correlates nicely with H-bond strength. This is the first detailed theoretical examination of substituent effects in H bonds and in these very closely related molecules, an electrostatic model works very well. The H-bond strength is directly proportional to the amount of *positive charge* the water oxygen sees as it approaches the ethanol O–H, as was also noted by Daly and Burton[18] in their study of hydronium and water ion H bonds. These model calculations also show that H-bond strength should increase with the electron-withdrawing power of the substituent, the 11α position being closer and more effective at causing this change.

(d) Relationship to Biological Activity. As stated in the introduction, it has been postulated that the 11β-hydroxy group is crucial to the biological activity of glucocorticoids, and that the enhancing effect of a fluorine atom at the 9α position is through increasing the strength of the 11β-hydroxy function as proton donor, which may be its role at the receptor site. The calculations confirm this effect in increasing the positive character of the hydroxyl proton.

Table II. Calculated Electron Density Differences at the Hydroxyl Proton of Substituted Cortisols

X-Ray coordinates used	Replacement of groups in X-ray coordinates[a]	$\Delta\rho$[b]
1. Cortisol	None	
2. Cortisol	6α-F	−0.002
3. Cortisol	9α-F	−0.006
4. 6α-Fluorocortisol	None	−0.003
5. 9α-Fluorocortisol	None	−0.006
6. 6α-Fluorocortisol	6α-H[a]	−0.001
7. 9α-Fluorocortisol	9α-H	−0.000
8. Cortisol	9α-OH	−0.003

[a] For example, the entry in row 6 means that the MO calculations were carried out using the X-ray data for 6α-fluorocortisol, substituting an H for the fluoro group (and appropriately scaling the bond distance). [b] Electron densities taken from the A and B ring closed structure (Figure 1b); for the B and C ring closed structure (Figure 1a), the entries in rows 4 and 5 would be −0.005 and −0.007, respectively.

It is of interest to inquire what effect an alternative enhancing fluorine substitution, *viz.*, the 6α-fluoro group, has on the acidity of the 11β-hydroxy group. It can be seen in Table II that for both 9α-F and 6α-F the proton becomes more acidic (rows 4 and 5), but this effect appears to be too small to explain completely the observed biological activity.[19] The H-bond energy difference calculated for a decrease in electron density at hydrogen of 0.011 was 0.94 kcal/mol (see Table I). Assuming little entropy differences between the H bond of cortisol and 9α-fluorocortisol and relating the electron density changes upon substituting 6α-F and 9α-F in cortisol to H-bond energies, one finds equilibrium constant ratios (relative to cortisol) of 2.3 for 9α-F and 1.5 for 6α-F. Thus, if the biological activity were solely related to H-bond strength the expected relative biological activities of cortisol, 6α-fluorocortisol, and 9α-fluorocortisol would be 1:1.5:2.3. That the observed activity differences are larger, and also that 0.94 kcal/mol is probably an upper limit to the relative H-bond strengths, represents ad-

(18) J. Daly and R. E. Burton, *Trans. Faraday Soc.*, 66, 2408 (1970).

(19) The electron density changes on the 11β-hydroxy proton are affected by the type of truncation employed (compare Figure 1a (A ring open) to Figure 1b (C ring open)). However, preliminary calculations on the steroids with three rings (A, B, and C) intact indicate no qualitative changes in the results presented in Table II.

ditional support for the conclusion that changes in the 11β-hydroxy acidity cannot completely explain relative glucocorticoid activities. Moreover, Devine and Lack[20] have shown that the hydrogen bonding ability of the hydroxyl proton of 9α-substituted 11β-hydroxyprogesterone derivatives decreases in the order F ≃ Cl > Br > H. If the cortisols behave similarly, there must be additional factors explaining the greater glucocorticoid activity of cortisol relative to 9α-bromocortisol.

Changes in the 11β-OH proton acidity will, however, affect processes other than hydrogen bonding. Thus Bush[21] has pointed out that by contrast to cortisol, which is extensively metabolized to the 11-ketone (cortisone), no C-11 ketone metabolites are found following administration of 9α-fluorocortisol. All of the metabolites are in the reduced form at C-11. This alteration in stability of the C-11 hydroxyl group must also be influenced by electron density changes in the C-11 OH group. Again, Wolff and Hansch[9] have found that the π parameter for 9α substituents is important in predicting biological activity. This is also evidence for an additional mechanism in activity enhancement, since the changes in π could have no discernible effect on the acidity of the 11β-hydroxy group.

Duax, *et al.*,[15] have shown that a major difference between the crystal structure of 9α-fluorocortisol and cortisol lies in the bowing of the A ring toward the α face of the substituted compound. As can be seen from the present electron density results, the largest density changes are in the A ring, but except for the changes at C-4, the shifts in the A ring of 9α-fluoro- and 6α-fluorocortisol are not necessarily in the same direction. These changes at C-4 may well represent the means by which C-9 substituents influence the stereochemistry and decrease of metabolic reduction at the C-4–C-5[21] double bond and could be responsible in part for the observed activity changes.

Although the geometrical distortion induced by 9α-substitution may also be responsible for an enhanced binding of the 3-keto group to the receptor, the changes seen in 6α-fluoro- and 9α-fluorocortisol relative to cortisol do not allow the conclusion that this effect alone explains the observed relative activities.

Conclusions

These calculations have enabled us to conclude that "long-range" effects in neutral steroids (either affecting reactivity or the proclivity for donor–acceptor complex formation) may be due mainly to conformation transmission (both *steric* and *electronic*). This situation is of importance in the interpretation of much of the kinetic and biological data on substituted steroids previously reported. In the case of the biological activity of corticoids, long-range effects between C-9 and C-4, and between C-6 and C-11, appear to be involved in the production of activity enhancing effects.

Interesting areas for future research will be X-ray and CNDO/2 examination of such electronegative substituents as 9α-bromo, 9α-hydroxy, and 9α-methoxy and such electron-donating substituents as 9α-methyl, all of which lead to only weakly active compounds. Another question raised by these results relates to the distortion of substrate bond angles and enzyme–substrate interactions[22] which has been advanced as a mechanism for enzymatic catalysis through stabilization of the transition state. It is clear that a CET mechanism could also be operative in this case and that the catalytic effect could be due not only to geometrical distortion but to changes in charge density at the reaction center which make the reaction proceed more rapidly.

(20) A. B. Devine and R. E. Lack, *J. Chem. Soc.* C, 1902 (1966).
(21) I. E. Bush and V. B. Mahesh, *Biochem. J.*, **69**, 9 (1958).
(22) For a discussion, see W. F. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 294–319.

A Proton Magnetic Resonance Study of the Conformations of 3′,5′-Cyclic Nucleotides

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Abstract: Complete analyses of the ^1H nmr spectra of the hormonal messenger 3′,5′-cyclic adenosine monophosphate, its dibutyryl derivative, and 3′,5′-cyclic thymidine monophosphate are presented. The ^{31}P–^1H and ^1H–^1H vicinal couplings are consistent with rigid phosphate and ribose ring structures. The phosphate rings are locked in the chair conformation, and the ribose rings are best described as 3′-endo-4′-exo (3′,5′-AMP and dibutyryl-3′,5′-AMP) and 4′-exo (3′,5′-TMP). The different biological activities of 3′,5′-AMP and its dibutyryl derivative cannot be ascribed to conformational differences in the ribose or phosphate rings. These conformations are consistent with those found in the solid state by X-ray crystallography. The measured ^1H–^{31}P couplings are used to formulate an expression for the dependence of such couplings upon dihedral angles.

Recently, considerable attention has been directed to the elucidation of the conformations of nucleosides and nucleotides by nuclear magnetic resonance spectroscopy.[2–4] This work has been facilitated by

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