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The Conformation of 20-Oxopregnane Hormones from Molecular Orbital Calculations and a Consideration of the Cortisol Receptor

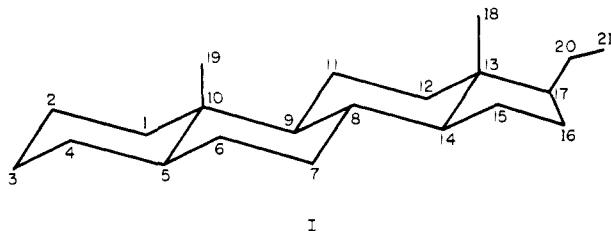
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Molecular orbital calculations have been performed on models representing the D ring and C-17 substituents of progesterone, corticosterone, and cortisol. The preferred conformations of these C-17 side chains have been deduced from the total energy minima derived from the calculations. The two-carbon side chain at C-17 was found to prefer a conformation in all three models in which the 20-oxo group projects toward the β face of the molecule and the C-20/C-21 bond eclipses the C-17/17 α H bond. In the cortisol molecule the intergroup distance between the 20-oxo and the 11 β -hydroxyl group was calculated to closely approximate the internitrogen distance previously calculated for histamine. The cortisol intergroup distance between the 3-oxo and 11 β -hydroxyl group was also found to approximate the internitrogen distance previously calculated for serotonin. A receptor complimentary pattern for cortisol is presented, based on the calculations, and its role as a possible antagonist in the inflammatory response is illustrated.

From a structural standpoint, nearly all of the positions and substituents of the pregnane hormones, such as progesterone and the adrenocorticoids, are easily defined because of the fused nature of the four rings. The only exception is the two-carbon keto side chain at C-17 which, in a conventional sense, is "free" to rotate to some extent. In the adrenocorticoids, the C-21 hydroxyl group destroys the symmetry of the methyl group of progesterone and thereby complicates prediction of the conformation of this ketol side chain. The biological activity of these steroids is markedly influenced not only by the C-21 hydroxyl group but also by a hydroxyl group in the C-17 α position. Any realistic approach to the mapping of a possible receptor for these steroid hormones must ultimately come to grips with the problem of assigning a preferred conformation to the side chain, whether it be an acetyl, α -ketol, or dihydroxyacetone grouping at C-17.



Numerous investigators have approached the problem of the side-chain conformation by considering the chemical reactivities of various derivatives of these steroids.¹⁻⁴ These efforts have centered mainly

around the reduction products of the 20-oxo group. It is certainly debatable whether the rates of reaction and relative yields of reduction products can be used to deduce the preferred conformation of the ground state of a molecule, since a perturbation in this conformation is necessarily introduced in passing through the transition states and reaction intermediates of the reaction. This particular perturbation would probably be significantly greater than the perturbations of a molecule experienced in the vicinity of its biological receptor.

Physical measurements have been used to deduce the 20-oxo group conformation. Djerassi has employed the "axial halo keto rule," with 17 α -halo-2-oxo derivatives, to assign a conformation to the acetyl side chain.⁵ Allinger has proposed a conformation of the side chain based on dipole-moment measurements.⁶

In recent years a method for the calculation of the preferred conformation of molecules using a theoretical approach based on quantum mechanics has been proposed by Hoffmann, and a fundamental justification of the conformational predictions has been presented.^{7,8} We have applied this theory to predict preferred conformations of phenylsydnone, acetylcholine, muscarine, muscarone, nicotine, histamine, ephedrine, pseudoephedrine, and serotonin.⁹⁻¹⁴ In every case, the preferred conformations determined by calculations of the total energy of the molecules have shown agreement with the conformations predicted from physical measurements (where available). This has

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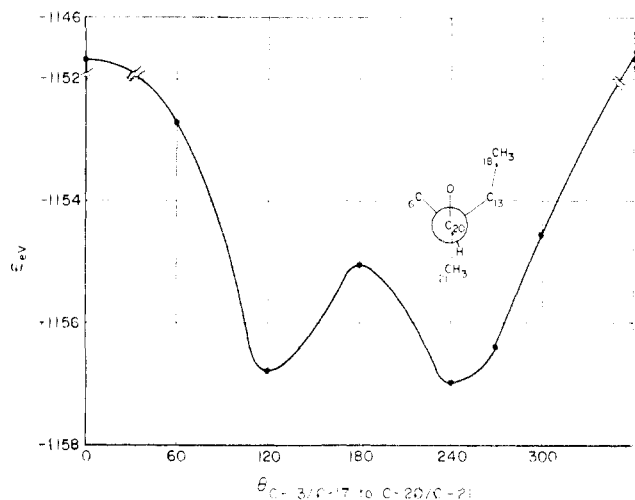


Figure 1.—Calculated energy *vs.* rotation of C-17/C-20 bond in model IIa.

encouraged us to consider that the energetically preferred conformations of these agonists may be pharmacologically significant and, if so, a guide to topology of the appropriate specific receptors. It is evident that such an approach to the steroids under discussion could possibly provide information leading to a better understanding of their hypothetical receptors.

Method

The extended Hückel theory (EHT) was used in the calculations with parameters previously employed.⁷ Since the program used for the computations permitted the treatment of only a limited number of atoms, it was necessary to simplify the model of the molecules under consideration by ignoring the perhydrophenanthrene portions of the steroids (rings A, B, and C).¹⁵ We therefore considered only the cyclopentane D ring of the three molecules studied with three variations at C-17, namely, as in IIa for progesterone, in IIb for corticosterone, and in IIc for cortisol. These simplifications are justifiable from the basic nature of conformational preference.^{16,17} The theoretical basis for the validity of our neglect of distant atoms is found in the appendix. Because of the mean distance between the C-17 side chain and the atoms omitted (rings A-C) (for example, the C-20 to C-11 and the C-20 to C-8 distance is more than 20 au), it seems reasonable to assume that the contribution of these distant atoms to the energy difference between any of the possible conformations of the side chain is quite small.

For these calculations (as in previous studies), we have selected bond-rotation angles at intervals of 60°. It is, of course, possible to refine the calculations by reducing these intervals; however, no real increase in accuracy would result in calculating the interatomic distances because of the error introduced by the assumption of standard bond lengths and angles in the calculation.¹⁸ Where an angle closer than 60° is

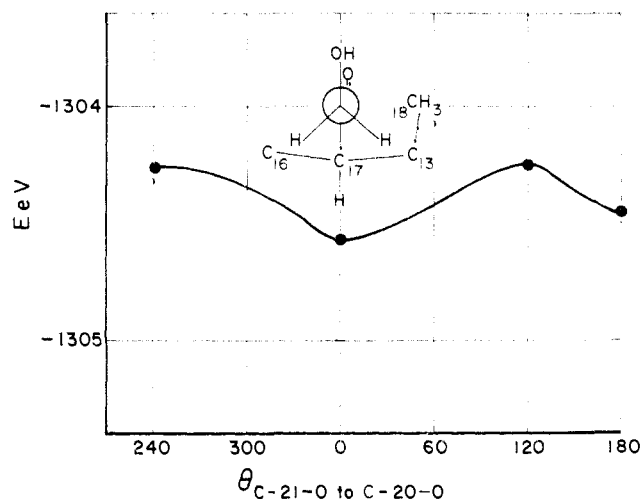
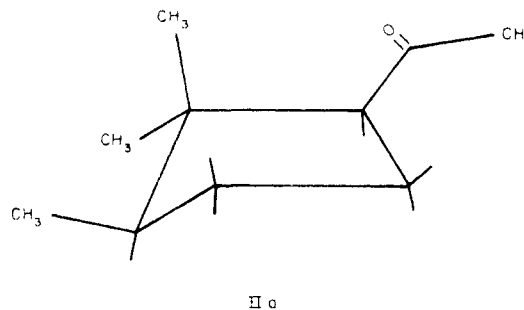


Figure 2.—Calculated energy *vs.* rotation of C-20/C-21 bond in model IIb.

desired, we have performed such a calculation. The conformation of the D ring was assumed to be the C₂ envelope with the C-14 below the plane of the other D-ring atoms.

Results

Pregnan-20-oxo Model.—The results of the calculations on this model, IIa, exemplifying progesterone, are displayed in Figure 1. A plot of the calculated total energy *vs.* rotation angle of the C-17/C-20 bond reveals that the minimum energy is associated with an angle of 240°. At this angle, the carbonyl group projects toward the β face of the steroid molecule and the C-20/C-21 bond eclipses the C-17 α hydrogen bond. The carbonyl oxygen is equidistant between the C-16 and C-13 atoms. There is a secondary preference for the 120° angle, but in this conformation the total energy of the molecule is about 0.2 eV higher than the energy of the 240° conformer. The second conformation with the C-17/C-20 bond at 120° places the C-20/C-21 bond in a position eclipsing the C-16/C-17 bond.



20-Oxo-21-hydroxy Model.—The results of the calculations on this model, IIb, with the α -ketol side chain, are shown in Figures 2 and 3. The relationship of the hydroxyl group to the oxo group is shown in Figure 2. The minimum energy is found for the conformation in which the C-OH bond eclipses the carbonyl oxygen bond. The energy minimum is found by rotating the C-17/C-20 bond to the 240° angle, which is the same as was obtained from the previous calcula-

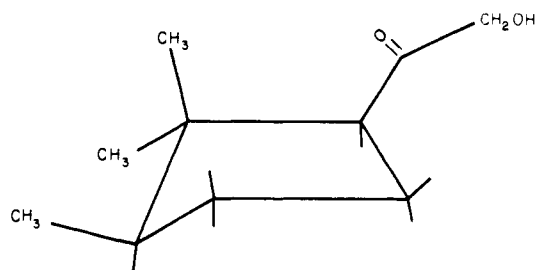
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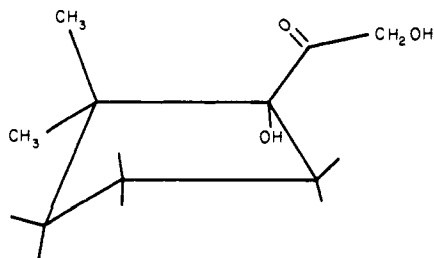
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tion when there was a C-17 acetyl side chain. The preferred side-chain conformation is thus a planar system, as shown in III.



II b

20-Oxo-17 α ,21-dihydroxy Model.—The results of the calculations on this model, IIc, are revealed in Table I



II c

TABLE I
VARIATIONS IN ENERGY WITH DIFFERENT CONFORMATIONS
OF THE C-17 AND C-20 HYDROXYL GROUPS

Conformation	Energy, eV	Structure
Type 1	-1348.538	
Type 2	-1348.147	
Type 3	-1348.147	
Type 4	-1348.098	

and Figure 4. In Table I, four relationships between the oxygen atoms are calculated and the energies are recorded. The conformation with the least energy has the C-21 hydroxyl group in the same conformation as was indicated by the previous calculation (based on the corticosterone model, IIb). It is interesting to note that the two possibilities of hydrogen bonding between the two hydroxyl groups are not favored energetically, although each of these conformations (types 2 and 3) are of equal energy and preferred over the conformation designated as type 4 in Table I. The energy difference between types 2 or 3 and type 4 then is a rough estimate of the hydrogen bond energy of the two possibilities and is calculated to be 1.15 kcal, a reasonable value. The preferred conformation (type 1) involving the three oxygen atoms is shown in IV.

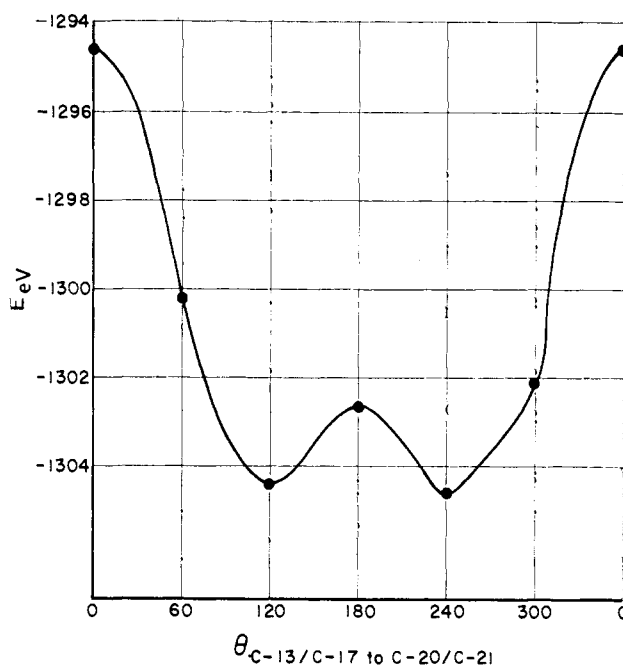
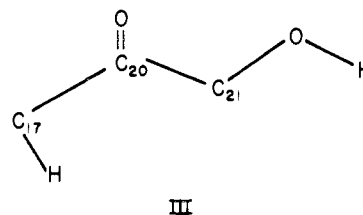
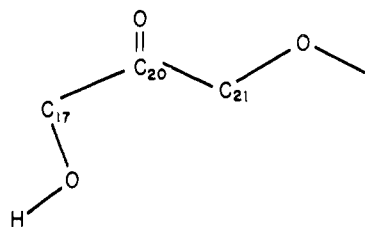


Figure 3.—Calculated energy vs. rotation of C-17/C-20 bond in model IIb.



III



IV

The calculations also reveal that the energy minimum occurs at the same angle of rotation of the C-17/C-20 bond, *i.e.*, at 240° in Figure 4, as was found in carrying out calculations based on models IIa and IIb representing progesterone and corticosterone, respectively.

Discussion

The calculations based on the three models, IIa-c, designed to simulate progesterone, corticosterone, and cortisol, respectively, reveal the same relationship between the 20-oxo group and the D ring in each instance. A comparison of our calculated results with those obtained from dipole moment experiments on progesterone show a fair agreement.⁶ According to Allinger's interpretation of his data, the preferred conformation would correspond to an angle of about 270° in Figure 1. Our calculations predict that the most energetically stable and therefore preferred conformation occurs when the bond angle is 240°.

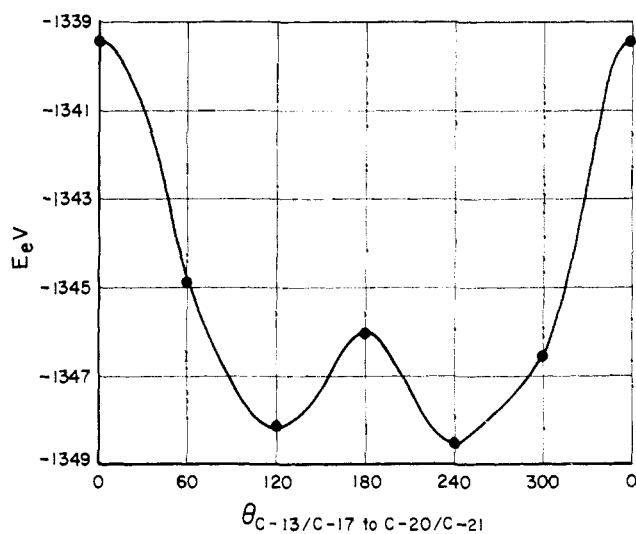


Figure 4.—Calculated energy vs. rotation of C-17-C-20 bond in model IIc.

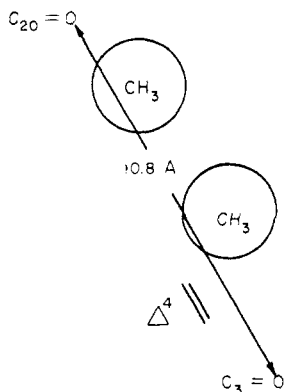


Figure 5.—Reflection of key atoms of progesterone (model IIa) in calculated preferred conformation.

With the results of these calculations in hand, we can now consider the three steroid molecules and the key interatomic distances in their preferred conformations. These patterns are illustrated in Figure 5 for progesterone, and in Figure 6 for corticosterone and cortisol. The pattern presented by cortisol is of interest due to the known antiinflammatory action of this compound.¹⁹⁻²²

There are two key interatomic distances in the cortisol molecule in its calculated preferred conformation (Figure 6). One distance is the approximately 4.8 Å separating the C-20 oxygen atom from the C-11 hydroxyl group. The second distance is the approximately 6.0-Å spacing between the C-30 oxygen atom and the C-17 hydroxyl group.

The significance of these proposed changes and distances in terms of a possible cortisol receptor remain to be seen. It is of interest, however, to note the similarity of these charges and distances to our previous calculations of the preferred conformations of histamine¹² and serotonin.¹⁴ It is possible to show how cortisol might interfere with both serotonin and histamine (Figure 7), which have been implicated,

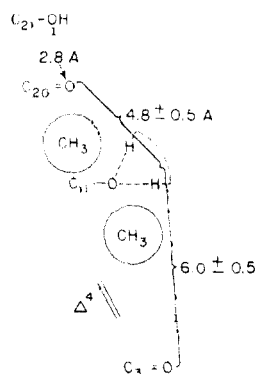


Figure 6.—Reflection of key atoms of corticosterone (model IIb) and cortisol (model IIc) in calculated preferred conformation.

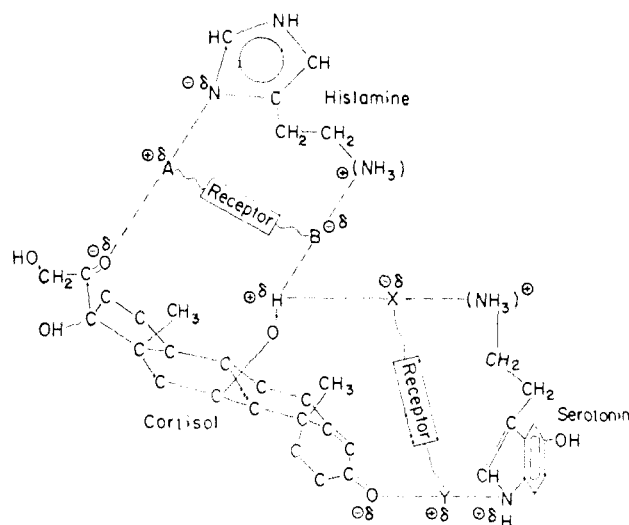


Figure 7.—Relationship of key atoms of cortisol in calculated preferred conformation to histamine and serotonin in their preferred conformations. A simultaneous engagement of the cortisol with the receptors is not intended to be implied.

among other substances, as inflammatory mediators.²³⁻²⁹ Cortisol is known to protect against, and to antagonize the results of, the intrusion of foreign substances into tissues.³⁰⁻³⁵

These calculated relationships between the interatomic distances in cortisol, a potent antiinflammatory drug, and histamine and serotonin do not necessarily prove that receptors for the latter two compounds are involved in the inflammatory process. It does offer an explanation as to how cortisol might function as an antagonist if these amine receptors were involved in directing the inflammatory response. In any event, a

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pattern of atoms has been calculated for the cortisol molecule which may reflect some features of the long sought steroid receptor(s).

Acknowledgment.—The author wishes to acknowledge helpful discussions with Dr. Michael Whitehouse.

Appendix

Proof Justifying Neglect of Distant Atoms.³⁶—It can be shown readily that one can neglect atoms in the molecule that are far away from the rotating group by application of the integral Hellman-Feynman theorem.³⁷ Consider a molecule in two conformations, denoted by x and y . The integral Hellman-Feynman theorem states that, if ψ_x and ψ_y are exact wave functions for these conformations

$$\Delta E = E_x - E_y = \frac{\int \psi_x (H_x - H_y) \psi_y d\tau}{\int \psi_x \psi_y d\tau}$$

where H_x and H_y are the Hamiltonians for the two conformations. Let $\Delta H = H_x - H_y$. ΔH contains only nuclear-electron attraction and nuclear-nuclear repulsion terms. Suppose that the molecule has n nuclei and the nuclei $1 - m$ are involved in the internal rotation and nuclei $m + 1 - n$ are fixed. Further, if internal rotation takes place (as is assumed) with fixed bond lengths, then the relative positions of nuclei $1 - m$ do not change. Then we can write

$$\Delta H = \sum_{i=1}^m \sum_{j=m+1}^n Z_i Z_j \left[\frac{1}{R_{ij,x}} - \frac{1}{R_{ij,y}} \right] - \sum_{k \text{ (all electrons)}} \sum_{i=1}^m Z_j \left[\frac{1}{r_{ki,x}} - \frac{1}{r_{ki,y}} \right]$$

where $R_{ij,x}$ is the distance between nuclei i and j in conformation x and $r_{ki,x}$ is the distance between electron k and nucleus i in conformation x . ΔE now becomes

$$\Delta E = \sum_{i=1}^m \sum_{j=m+1}^n Z_i Z_j \left[\frac{1}{R_{ij,x}} - \frac{1}{R_{ij,y}} \right] - \frac{\sum_{i=1}^m Z_i \int \psi_x \left[\sum_k \left(\frac{1}{r_{ki,x}} - \frac{1}{r_{ki,y}} \right) \right] \psi_y d\tau}{\int \psi_x \psi_y d\tau}$$

The wave functions ψ_x and ψ_y can be written approximately as antisymmetrized products of localized MO's, $\phi_1 - \phi_i$. *Ab initio* calculations have shown that the localized MO's (which correspond closely to bond orbitals) whose centroids of density are removed

from the rotating group by more than about 5-6 Å do not change on rotation. (The centroid of density for bonds that are not highly polar can be taken to be the midpoint between the two atoms between which the orbital is localized.)

We partition the MO's into groups. Let $\phi_1 - \phi_r$ represent bonds far from the rotating group. We then write ψ_x and ψ_y as the Slater determinants

$$\psi_x = |\phi_1 \bar{\phi}_1 \dots \phi_r \bar{\phi}_r \phi_{r+1}' \dots \bar{\phi}_i'|$$

$$\psi_y = |\phi_1 \bar{\phi}_1 \dots \phi_r \bar{\phi}_r \phi_{r+1}'' \dots \bar{\phi}_i''|$$

where a bar indicates β spin and no bar indicates α spin. The orthogonality relationships are $\int \phi_i \phi_j d\tau = \delta_{ij}$ for all i, j ; $\int \phi_i \phi_j d\tau = \int \phi_i \phi_j'' d\tau = 0$ for all i, j ; $\int \phi_i' \phi_j'' d\tau = S_{ij}$; and S_{ij} in general is not equal to zero. Using these orthogonality relationships and the usual quantum mechanical rules for the construction of matrix elements between Slater determinants, we can write

$$\frac{\sum Z_i \int \psi_x \left[\sum_k \left(\frac{1}{r_{ki,x}} - \frac{1}{r_{ki,y}} \right) \right] \psi_y d\tau}{\int \psi_x \psi_y d\tau} = -2 \sum Z_i \left[\sum_{j \neq i} \int \phi_{j(\alpha)} \left(\frac{1}{r_{1i,x}} - \frac{1}{r_{1i,y}} \right) \phi_{j(\alpha)} d\tau \right]$$

plus other terms involving only the orbitals ϕ_k' and ϕ_k'' . Assuming that the centroids of density of the ϕ_j lie at the bond midpoints and using the fact that these points are very far from the rotating nuclei, we can replace the integration by a simple sum of inverse distances from these points to the rotating nuclei

$$-2 \sum_i Z_i \left[\int \phi_{j(\alpha)} \left(\frac{1}{r_{1i,x}} - \frac{1}{r_{1i,y}} \right) \phi_{j(\alpha)} d\tau = -2 \sum_{K \text{ (bonds far from rotating nuclei)}} \sum_i Z_i \left(\frac{1}{R_{ki,x}} - \frac{1}{R_{ki,y}} \right) \right]$$

where R_{ki} is the distance from nucleus i to the midpoint of bond k . Using the fact that $2R_{ki}^{-1}$ is, for these large distances, very nearly equal to the sum of the inverse distances from the two nuclei being bonded to the rotating nucleus i , it is seen that, adding up all contributions, the sum effectively cancels that part of the nuclear-nuclear repulsion term involving those centers far from the rotating part of the molecule. Since these nuclear-nuclear terms are small to begin with, and are then further reduced essentially to zero, it is quite reasonable to drop them from consideration in computing the rotational barriers.

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