surface of the myelin vesicles in our experiments bears a significant resemblance to the myelin membrane in vivo and the interaction of MBP and myelin is largely electrostatic, then anything that perturbs the charge or charge density of the membrane will lead to decreased binding of MBP. In the present case, the charge is perturbed by the protonation of protein components. In vivo, perturbation could result from a number of mechanisms including enzyme-catalyzed cleavage of anionic residues from membrane phospholipids or sulfatides. This suggests that the primary events in inflammatory demyelination may be associated with increases in these enzyme activities. A simpler mechanism of destabilization of the myelin lamellae would involve a simple pH effect, such as seen in Figure 6. Lysosomal granules, at about pH 3–4, lysed in the immediate vicinity of the sheath would provide a high local concentration of lactic and other small organic acids. Diffusion of these across the lamellae would drastically lower the pH within the confines of the major density region, allowing the lamellae to separate. This type of internal "splitting" along major densities is often observed in electron micrographs during the phagocytosis of myelin.[23]

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Molecular Structure of 18-Deoxyaldosterone and Its Relationship to Receptor Binding and Activity[1]

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Abstract: 18-Deoxyaldosterone possesses one-third the binding affinity of aldosterone for the cytoplasmic mineralocorticoid receptor and exhibits an approximate 2:1 antagonist to agonist ratio. Crystals of 18-deoxyaldosterone contain two molecules that differ significantly from one another in 17β-side-chain orientation, 4-en-3-one conjugation, and hydrogen bonding. The C(20)–O(20) bond is synperiplanar to C(17)–C(16) in molecule I and to C(17)–C(13) in molecule II. The latter conformation, previously observed only in the presence of a 16β-methyl or -halo substituent, has been stabilized by the fact that epoxide formation draws the C(18) methyl away from the D ring. The two conformers must be in equilibrium in solution. Both molecules of 18-deoxyaldosterone resemble aldosterone in the overall shape of the A, B, C, and E rings. Molecule II and aldosterone have similar hydrogen bonding to O(3) and nearly planar 4-en-3-one conformations. Although the D-ring composition of aldosterone and 18-deoxyaldosterone is different, the side-chain orientation of molecule I of 18-deoxyaldosterone comes closest to approximating that of aldosterone in shape and potential hydrogen-bonding geometry. Analysis of the conformations and activity of aldosterone, 18-deoxyaldosterone, and spironolactone is in agreement with the model which proposes that the A-ring end of the steroid is primarily responsible for initiating and maintaining receptor binding and D-ring variation governs agonist–antagonist response. Molecule II appears to have an A ring ideally suited to receptor binding and a side-chain orientation that would elicit little or no subsequent activity, while molecule I has the side-chain orientation that most likely contributes to the partial agonism exhibited by the molecule. The crystal structure of the hemihydrate of 18-deoxyaldosterone (a = 19.878 (3) Å, b = 30.341 (4) Å, c = 5.9951 (5) Å, $P2_12_12_1$) was determined by direct methods and refined to a final R index of 0.072.

18-Deoxyaldosterone (21-hydroxy-11β,18-oxido-4-pregnene-3,20-dione) is an analogue of the natural mineralocorticoid hormone aldosterone in which the aldehyde hemiacetal structure is replaced by a stable 11β,18-oxide ring (Figure 1). Removal of the 18-oxo group from aldosterone transforms it from an agonist into an antagonist. The 18-deoxy derivative possesses one-third of the binding affinity of aldosterone for the cytoplasmic mineralocorticoid receptor and exhibits an approximate 2:1 antagonist to agonist ratio in toad bladder and adrenalectomized rat bioassay systems.[4]

An equilibrium between the structural isomers of Figure 2 has been proposed on the basis of chemical studies of aldosterone. Because isomers b and c introduce two new chiral centers into the structure, a total of seven isomers are possible. X-ray analysis of the only stable crystal form of aldosterone revealed it to be a monohydrate of the 18(R)-acetal-20(S)-hemiketal isomer (c).[5]

Removal of the 18-hydroxy group from the hemiacetal form of aldosterone would be expected to relieve some of the strain observed in the 18(R)-acetal-20(S)-hemiketal isomer.[5] Nevertheless it is difficult to predict how this change will affect the corticoid side-chain orientation and the overall shape of the molecule. The X-ray crystal structure of 18-deoxyaldosterone was undertaken in order to compare the conformation with that of aldosterone and to attempt to define the structural basis for the difference in binding affinity and activity between the two steroids.

Results

Crystals of 18-deoxyaldosterone contain two crystallographically independent molecules that differ significantly from one another

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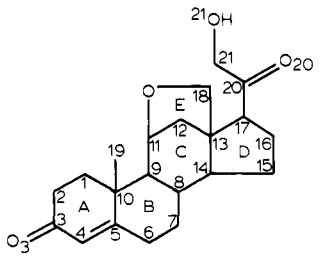

Figure 1. Atomic numbering and ring nomenclature for 18-deoxyaldosterone, 21-hydroxy-11β,18-oxido-4-pregnene-3,20-dione.

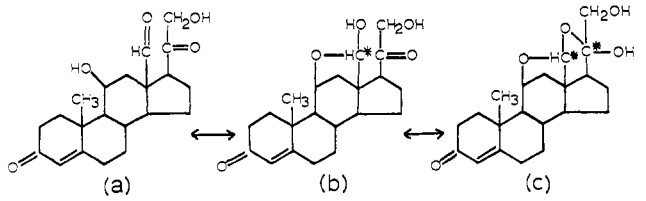

Figure 2. Aldosterone structural isomers include (a) one 18-aldehyde, (b) two 11β,18-oxides differing in configuration at C(18), and (c) four 18-acetal-20-hemiketals differing in configuration at C(18) and C(20).

Table I. Hydrogen Bonds in the Hemihydrate of 18-Deoxyaldosterone

donor–acceptor	Å
O(21) (I)–O(3) (II)	2.716
O(w)–O(3) (II)	2.97
O(w)–O(21) (II)	2.70
O(21) (II)–O(21) (I)	2.724

in the orientation of the 17β side chain. Stereo views[6] of the two molecules are presented in Figure 3. The orientation found in molecule I is the typical one observed in 81 of 85 steroid structures having a 20-one substituted side chain.[7] The side chain in molecule II takes up a conformation that has previously been observed only in 16β-substituted pregnanes. This conformational change is accompanied by significant bond-length and angle changes. The D ring has a 14α-envelope conformation in molecule I and a 13β-envelope conformation in molecule II.[8] When the carbonyl bond nearly eclipses the C(13)–C(17) bond in molecule II, steric crowding causes the C(18)–C(13)–C(17), C(13)–C(17)–C(20), and C(17)–C(20)–O(20) angles to expand to 118°, 120.3°, and 123.7° from 115.9°, 112.5°, and 120.0°, respectively, in molecule I.

The two molecules of 18-deoxyaldosterone differ in the conformation of their A and B rings as indicated by the torsion angles of Figure 4. The torsion angles of aldosterone are also included for comparison. These differences are probably due to a combination of factors, including long-range conformational effects related to the side-chain and D-ring changes, differences in hydrogen bonding to O(3), and crystal-packing forces. The conformational change in the D ring can be transmitted to the B ring by interaction between the hydrogens on C(15) and C(7). The O(3) atom accepts two hydrogen bonds in molecule II and none in molecule I (Table I). The hydrogen-bonded carbonyl is more nearly coplanar with the C(4)–C(5) bond and exhibits ideal trigonal geometry at C(3) with a shorter C(3)–C(4) bond.

In its monohydrate crystal form aldosterone has a similar A-ring conformation and hydrogen bonding. Because the C ring and the additional ring generated by epoxide formation (ring E) have nearly identical conformations in the two independent molecules and in aldosterone, they provide a frame of reference for comparison of differences in the overall shape of the molecules (Figure 5).[9] The B, C, and E rings of molecule I and aldosterone are nearly indistinguishable. Although the differences between molecule II and aldosterone are greater, they have in common the 4-en-3-one geometry and hydrogen bonding to O(3).

The 21-hydroxyl acts as a hydrogen-bond donor and acceptor in molecules I and II of 18-deoxyaldosterone and in aldosterone. However, the direction of these hydrogen bonds is quite different as a natural consequence of the differences in C(21)–O(21) bond orientation in the three molecules (Figure 5). The O(20) carbonyl of 18-deoxyaldosterone does not participate in any hydrogen bonding, whereas the O(20) hydroxyl of aldosterone acts as a hydrogen-bond donor and acceptor.

Discussion

Analysis of CD spectra of 16β-methyl-substituted progesterone[10] indicates that the two side-chain conformations observed in crystal structures[7] are of approximately equal energy. Steric interaction between the 16β-methyl and the O(20) substituent is the driving force for stabilizing the conformer in which the C(20)–O(20) bond is synperiplanar with C(13)–C(17). In the structure of 18-deoxyaldosterone, the epoxide formation draws C(18) away from the side chain, thus removing steric interference with the secondary conformation. Although the D-ring conformational change is clearly correlated with the change in side-chain orientation and the A-ring conformation appears to be directly correlated with the absence or presence of hydrogen bonding to O(3), it is difficult to determine whether there is long-range correlation between these structural changes.

On the basis of structural studies of progestins[11] and estrogens,[12] we have previously proposed that the A-ring end of these steroids has primary responsibility for initiating and maintaining receptor binding and the D-ring end of the molecule has its greatest impact upon activity, either by stabilizing a conformational change in the receptor or participating in a macromolecule interaction subsequent to receptor binding. A comparison between aldosterone and the antimineralocorticoid spironolactone suggested that such a partitioning of steroid function might also apply to mineralocorticoids. Spironolactone and aldosterone have similar A-ring conformations and differ significantly in the shape and hydrogen-bonding ability of their D-ring substituents. However, the fact that removal of the 19-methyl converts progesterone from a mineralocorticoid antagonist to an agonist[13,14] indicates that this compartmentalization of binding and activity requires further refinement.

Since aldosterone and 18-deoxyaldosterone compete for the same site on the receptor,[4] the reduction in binding and antagonistic properties of the latter must be primarily associated with the structural variation at the D-ring end of the molecule. Nevertheless the D ring variation could also have a transmitted effect upon the binding capacity of the A-ring. If 18-deoxyaldosterone exists in an equilibrium between the conformers observed here, perhaps only molecules with the conformation of molecule I compete for receptor binding due to the similarity between its A-ring conformation and hydrogen bonding and that of aldosterone. An equilibrium between species that do and do not bind would account for the lower affinity of 18-deoxyaldosterone.

The antagonist properties of 18-deoxyaldosterone are almost certainly a result of the reduction of hydrogen-bonding capacity of the 17 side chain when compared to aldosterone regardless of conformation. Although the 20-carbonyl of 18-deoxyaldosterone

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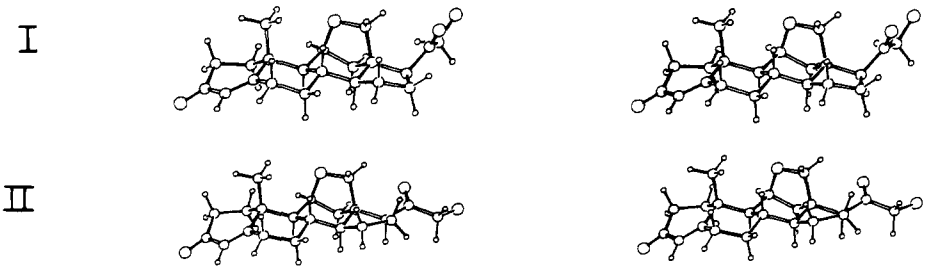

Figure 3. Stereo ORTEP views of the two molecules of 18-deoxyaldosterone illustrating difference in 17β side-chain orientation.

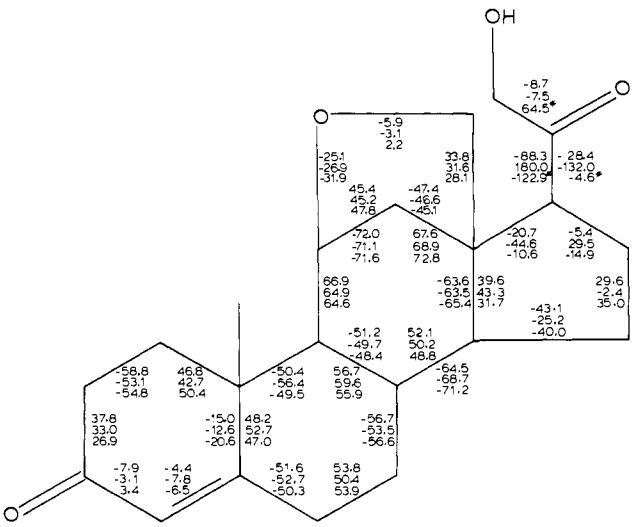

Figure 4. Intraring torsion angles for molecule I (top) and II (middle) of 18-deoxyaldosterone compared with those of aldosterone (bottom). A torsion angle α–β–γ–δ is positive if when viewed down the β–γ bond the α–β bond will eclipse the γ–δ bond when rotated less than 180° in a clockwise direction. The asterisk indicates that the side chain of aldosterone has a different constitution.

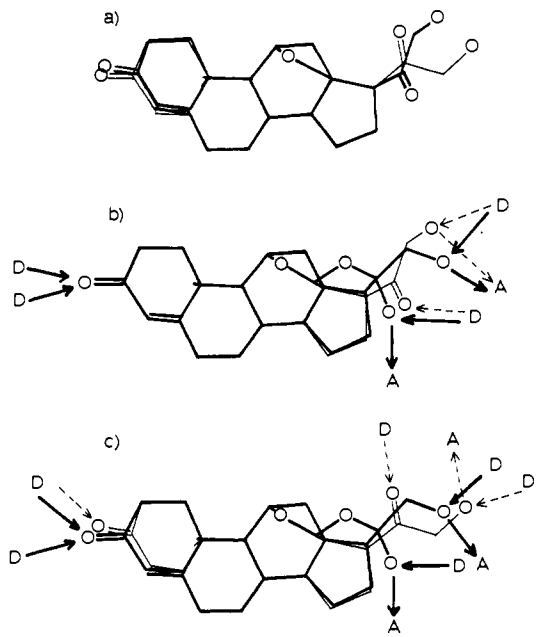

Figure 5. Comparison of crystallographically observed conformations of 18-deoxyaldosterone and aldosterone after least-squares fitting of the C and E rings of (a) the two molecules of 18-deoxyaldosterone, (b) molecule I and aldosterone, and (c) molecule II and aldosterone. The observed direction of the location of hydrogen-bond donors (D) and acceptors (A) is indicated. Molecule I is darker in (a) and aldosterone is darker in (b) and (c). Dark lines are used to indicate hydrogen bonds to aldosterone and dashed lines indicate hydrogen bonds to 18-deoxyaldosterone.

could act as a hydrogen-bond acceptor it does not do so in either of the molecules in the crystal. In contrast, the 20-hydroxyl of aldosterone can and does act as both a donor and acceptor of hydrogen bonds. While the antagonist behavior of progesterone could also be ascribed to the absence of hydrogen-bond donor capabilities at its D-ring end, the agonist activity of 19-norprogesterone would require some further explanation. It may be that 19-methyl removal enhances A-ring affinity for the receptor to such an extent that this compensates for reduced D-ring potential for hydrogen-bond formation necessary for receptor activation. The side-chain conformation of molecule I of deoxyaldosterone roughly approximates that of aldosterone (Figure 5b) and is the likely candidate to explain the partial agonist behavior of the compound. In this position O(21) of aldosterone and 18-deoxyaldosterone accept and donate hydrogen bonds from roughly the same direction and the O(20) carbonyl of 18-deoxyaldosterone could act as a hydrogen-bond acceptor similar to the O(20) hydroxyl of aldosterone. If these superpositions were correct, the side-chain orientation that most nearly approximates conditions needed for activity would occur in combination with the A-ring shape inappropriate to high-affinity binding. Thus, only half the molecules of 18-deoxyaldosterone would be suited to receptor binding and only a fraction of those would have a side-chain conformation that meets most of the requirements for activity.

Experimental Section

Single crystals of 18-deoxyaldosterone hemihydrate [$C_{21}H_{28}O_4 \cdot 0.5H_2O$] were crystallized from 50% methanol. Lattice parameters (a = 19.878 (3) Å, b = 30.341 (4) Å, c = 5.9951 (5) Å, V = 3615.8 Å^3, M_r = 362.4, Z = 8, D_c = 1.298 g cm^{-3}, space group $P2_12_12_1$) were determined from least-squares fit to 21 reflections with 60° < 2θ < 70°. The intensities of 2905 independent reflections with 2θ < 115° were measured on a Syntex P3 diffractometer with Cu Kα radiation; 2049 of these reflections were measured to be above background ($F > 2\sigma_F$). The structure was solved by direct methods[15] and refined by full-matrix least-squares procedures. Hydrogen atoms were placed at their geometrically expected positions (C–H distances = 1.08 Å, normal tetrahedral angles) and included in the structure factor calculations for the final least-squares cycles although their positions were not refined. Non-hydrogen atoms were refined anisotropically. The final R index was 0.072 for the 2049 reflections, with $|F_o| > 2\sigma_F$ and 0.103 for all data.

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Registry No. 18-Deoxyaldosterone, 2507-88-2.

Supplementary Material Available: Table of atomic coordinates and anisotropic thermal parameters, and figures of bond distances and angles for the non-hydrogen atoms and of crystal packing (3 pages). Ordering information is given on any current masthead page.

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