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Relationships of the Molecular Structure of Aldosterone Derivatives with Their Binding Affinity for Mineralocorticoid Receptor

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SUMMARY

The molecular structures of 19-nor-11-deoxycorticosterone **(III)** and 21-hydroxypregna-4,11-diene-3,20-dione **(IV)** were determined by X-ray crystallographic analysis and the factors affecting the binding affinities for the mineralocorticoid receptor were examined with six aldosterone derivatives **(I–VI)** containing these two compounds. The most important factor was found to be the steric one; affinity increased with increasing flatness of the structure. The electronic factor may be a minor influence although a good relationship was found between the affinity and the ^{13}C-NMR chemical shift of the C(5) atom. The factor playing no role in the binding is the hydrophobic one.

The binding of steroids to steroid hormone receptors is mainly dominated by hydrophobic, steric, and electronic factors, each of which plays the main role in a different stage. When a steroid molecule is transported to the active site in the receptor through hydrophobic envelopment from a hydrophilic phase, such as in an aqueous solution or when the active site is hydrophobic, the hydrophobicity of the molecule becomes an important factor in the binding to the receptor. How well the molecule fits the active site is closely related to its three-dimensional structure, that is, its steric feature. In the active site, the A ring and the D ring of the steroid interact with the corresponding areas at the site through electronic and/or hydrogen bonding interactions. According to Duax and co-workers (1–3), the A ring is the most important for the initiation of the binding to the receptor, whereas the D ring primarily influences the expression of mineralocorticoid, progestin, and estrogen hormone actions. Finally, the steroid-receptor complex is transformed into the conformer which can translocate to the nucleus of the cell in order to induce the hormone action.

One of the most important factors responsible for the binding is the conformational feature of the steroids. The correlation between the degree of flatness of the A ring with the 4-en-3-one system of the steroids and the degree of binding affinity for steroid receptors has well been discussed in the case of androgen and progestin receptor bindings (Ref. 4 and references cited therein). The examples used were 19-nor derivatives and the derivatives with estra-4,9-dien-3-one or estra-4,9,11-trien-3-one functions which exhibit both high *in vitro* and *in vivo* androgenic and progestational activities.

An apparent correlation also exists between the strength of the binding affinity for the MR or the potency of *in vivo* mineralocorticoid activity and the degree of flatness of conformations of several naturally occurring mineralocorticoid hormones, such as corticosterone **(I)**, 11-deoxycorticosterone **(II)**, 19-nor-11-deoxycorticosterone **(III)**, and aldosterone **(VI)**, and synthetic 18-deoxyaldosterone **(V)**. Strong receptor binding and high *in vivo* mineralocorticoid activity also seem to appear after removal of substituents which would cause bending of the A ring toward the α-face (e.g., 19-methyl or 11β-hydroxy group) and also after formation of an ether bridge or an acetal bridge which would induce the molecule into a flat conformation.

Predicting that a similar conformational effect would occur upon introduction of a double bond between the C(11) and C(12) positions, we synthesized 21-hydroxypregna-4,11-diene-3,20-dione **(IV)**. We then measured its binding affinity for the MR and its *in vivo* mineralocorticoid activity and compared them with those of other mineralocorticoids (5).

The molecular conformations of the newly synthesized compound **IV** and the already known compound **III** were determined by X-ray crystallographic analysis and compared with the known conformations of the other mineralocorticoids, **I, II, V,** and **VI** (1, 6–8). Their infrared and ^{13}C-NMR spectra were measured in order to obtain information on the electronic structure of the A ring and the D ring of the steroids. Their

ABBREVIATIONS: MR, mineralocorticoid receptor; r-TLC, reverse phase thin layer chromatography; RBA, relative binding affinity.

TABLE 1
Crystallographic details for III and IV

	III	IV
Molecular formula	$C_{20}H_{28}O_3$	$C_{21}H_{28}O_3$
Molecular weight	316.44	328.45
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
Cell dimensions		
a (Å)	12.784(1)	10.587(1)
b	13.273(1)	11.623(1)
c	10.361(1)	7.438(1)
β (degrees)	90.0	103.91(1)
V(Å^3)	1758.0(3)	888.4(2)
Z	4	2
Crystal size (mm)	0.4 × 0.2 × 0.05	0.2 × 0.2 × 0.2
Density calculated (g/cm^3)	1.20	1.23
Radiation (graphite monochromated)	CuK_α	CuK_α
θ_{max} (degrees)	65.0	70.0
Scan mode	ω-2θ	ω-2θ
No. of reflections		
Unique	1720	1723
Observed [$\|F_o\| \geqq \sigma_1(F_o)$]	1599	1699
R	0.040	0.031
R_w	0.059	0.044
No. of reflections used for least squares refinement	1423	1598

hydrophobicities were evaluated using an r-TLC method. Based on these experimental results, the factors which govern the binding of these six steroids for MR were examined.

Experimental Procedures

Materials. Details of all the steroid compounds used have been described elsewhere (5, 9).

X-ray crystal analysis. Crystals of **III** and **IV** were obtained from the respective MeOH solutions. Cystallographic details are listed in Table 1. Cell dimensions were determined by least squares fit of the 2θ values for 20 reflections (35° ≦ 2θ ≦ 45°) centered on a Rigaku AFC-5 diffractometer. During data collection, 3 standard reflections were monitored for every 100 reflections, which showed no significant change in their intensities. All intensities were corrected for Lorentz and polarization factors, but not for absorption effects.

The structures were solved using the program MULTAN 78 (10). All hydrogen atoms, except that of the hydroxyl group in **III**, were located on the respective difference electron density maps. The positional parameters of all of the atoms and the anisotropic thermal ones of the non-hydrogen atoms were refined by the block-diagonal least squares method. The temperature factor of each hydrogen atom was assumed to be isotropic and equal to B_{eq} (*cf.* Footnote *a* in Table 2) of the bonded atom. All of the hydrogen atoms had converged to positions not far from those expected on chemical grounds, with the C-H bond distances ranging from 0.90 to 1.17 Å. The final parameter shifts were sufficiently small compared with the estimated standard deviations of the parameters. The function minimized in the refinement was $\Sigma(w|\Delta F|^2)$. The weighting scheme was $w = 1/\sigma^2(F_o)$ for the reflections with $|F_c| \geqq \sigma(F_o)$ and $|\Delta F| \leqq 3\sigma(F_o)$, and $w = 0$ otherwise. $\sigma(F_o)$ was estimated as $[\sigma_1{}^2(F_o) + c^2|F_o|^2]^{1/2}$, where $\sigma_1(F_o)$ is the estimated standard deviation depending on the counting errors and c^2 is 0.002256 for **III** and 0.001593 for **IV**.

Spectroscopic measurement. Infrared and ^{13}C-NMR spectra of the compounds were measured using JASCO A-702 and Varian XL-100-12A spectrometers, respectively.

Evaluation of the hydrophobic character. The hydrophobic parameter was obtained by two methods: an r-TLC and a calculation method. In the r-TLC method (11), 1 μl of acetone solution containing 1 mg/ml of a compound was spotted on a high performance thin layer chromatographic plate, RP-2 F_{254}, for nano-thin layer chromatography (Merck), and an aqueous solution with various proportions of acetone (45–60%) was used as the mobile phase. The compound was detected under UV light. The interpolated R_m value at 50% acetone was adopted. In the calculation method, the log P value was evaluated by the CLOGP-3 program (12, 13).

Relative binding affinity for MR (RBA). The RBA was determined by a competitive binding assay. Rat renal cytosol was obtained from kidneys of male Sprague-Dawley rats (4–8 weeks old) which had been adrenalectomized bilaterally 1 or 2 days earlier. The cytosol was incubated with various concentrations of steroids and about 1 nM ^{3}H-aldosterone (Amersham International) for 42–48 hr at 0°C. Bound and free steroids were separated by the dextran-coated charcoal method. The RBA was determined from: RBA (%) = 100 × $^{50}_0$C/^{50}C, where $^{50}_0$C and ^{50}C are concentration of aldosterone and the competitor, respectively, at 50% inhibition of ^{3}H-ligand bindings of control. Further details have been described elsewhere (5, 9).

Results

Atomic coordinates are listed in Table 2. Tables of atomic coordinates for hydrogen atoms, anisotropic thermal parameters, bond lengths, and angles are available upon request (M. S.).[1]

Perspective views of the molecules of **III** and **IV** are presented in Fig. 1. The rings of A–C, respectively, adopt a sofa [C(1) flapped], chair, and chair conformation in **III**, and a sofa [C(1) flapped], chair, and half-chair one in **IV**, although they are somewhat distorted. The structures of the D ring including the hydroxyacetyl group are almost common in **II, III,** and **IV:** the ring adopts a half-chair conformation midway between a 13β envelope and a 14α envelope; the hydroxyacetyl group in which the hydrogen bond is formed between the hydroxyl and carbonyl oxygen atoms is approximately planar; and the torsion angle of C(16)—C(17)—C(20)—O(20) is −11.2° for **II**(7), −16.3° for **III**, and −14.5° for **IV**. The bond lengths of C(9)—C(10) [1.557 (4), 1.564 (2) Å] and C(13)—C(17) [1.579 (3), 1.564 (2) Å] in **III** and **IV** are significantly longer than the normal value expected for C(sp^3)—C(sp^3), 1.533 Å, similar to the findings with **II** [1.564, 1.560 Å] (7). None of the other bond lengths are unusual. In the crystals of **III** and **IV**, intermolecular hydrogen bonds are not formed.

The R_m and the calculated log P value are cited in Table 3, and a good relationship was found between them as shown in Eq. 1:

$$\log P = 7.12R_m - 0.079 \ (n = 6, r = 0.958, s = 0.409) \quad (1)$$

where n is the number of data points used in the regression, r the multiple correlation coefficient, and s the standard deviation.

Biagi *et al.* (11) pointed out that the R_m values are satisfactorily correlated with the observed log P value in a steroid series. Therefore, in our case, adopting the calculated log P value, as well as the R_m value, as one of hydrophobic parameters seems to be reasonable.

Discussion

We examined the effects of steric, electronic, and hydrophobic factors on the binding affinity of steroids for the MR.

Steric effects. The steric factor was the most important in the steroid-receptor binding in the cases studied. Comparison of the RBA in Table 3 with the molecular structures in Fig. 2 showed that the RBA increases with increasing flatness of the molecular structure. Of the six steroids in Table 3, the RBA

[1] Correspondence should be addressed to M. Y., M. S., and S. K. for QSAR analysis, X-ray crystallographic analysis, and organic chemistry, respectively.

TABLE 2

Atomic fractional coordinates (×10⁴) and equivalent isotropic temperature factors (×10²), B_{eq}, with their estimated standard deviations in parentheses

	III				IV			
	x	y	z	B_{eq}*	x	y	z	B_{eq}
C(1)	8,308(2)	4,851(3)	8,297(4)	732(9)	5,911(2)	3,000	10,458(2)	394(4)
C(2)	9,507(2)	4,882(3)	8,467(5)	803(9)	7,133(2)	3,716(2)	11,221(2)	461(4)
C(3)	10,000(2)	5,489(3)	7,412(3)	660(9)	7,908(2)	3,851(2)	9,798(3)	465(4)
C(4)	9,407(2)	6,345(3)	6,903(3)	615(9)	7,171(2)	3,920(2)	7,873(3)	450(4)
C(5)	8,419(2)	6,556(2)	7,282(2)	510(7)	5,876(1)	3,797(1)	7,312(2)	359(3)
C(6)	7,900(2)	7,509(2)	6,815(3)	584(8)	5,207(2)	3,979(2)	5,307(2)	492(5)
C(7)	6,737(2)	7,344(2)	6,460(2)	538(8)	4,222(2)	3,030(2)	4,530(2)	465(4)
C(8)	6,178(2)	6,875(1)	7,593(2)	405(6)	3,278(1)	2,906(1)	5,767(2)	321(3)
C(9)	6,648(1)	5,842(2)	7,917(2)	412(6)	4,033(1)	2,571(1)	7,737(2)	300(3)
C(10)	7,838(2)	5,919(2)	8,221(2)	478(7)	5,038(1)	3,502(1)	8,660(2)	313(3)
C(11)	6,066(2)	5,327(2)	9,027(3)	465(6)	3,131(1)	2,196(2)	8,929(2)	354(3)
C(12)	4,873(2)	5,269(1)	8,818(2)	438(6)	1,872(1)	1,951(2)	8,297(2)	360(3)
C(13)	4,427(1)	6,317(1)	8,541(2)	372(5)	1,182(1)	2,106(1)	6,297(2)	296(3)
C(14)	4,992(2)	6,733(1)	7,361(2)	396(5)	2,201(1)	2,016(1)	5,140(2)	314(3)
C(15)	4,333(2)	7,635(2)	6,947(3)	586(9)	1,407(2)	1,967(2)	3,139(2)	427(4)
C(16)	3,205(2)	7,263(2)	7,150(3)	631(9)	200(2)	1,264(2)	3,265(2)	414(4)
C(17)	3,269(2)	6,319(2)	8,010(2)	410(5)	225(1)	1,138(1)	5,348(2)	330(3)
C(18)	4,500(2)	6,999(2)	9,728(2)	502(7)	463(2)	3,261(2)	6,077(3)	441(4)
C(19)					4,349(2)	4,603(2)	9,074(3)	462(4)
C(20)	2,456(2)	6,305(2)	9,047(2)	452(6)	−1,116(1)	1,187(2)	5,748(2)	390(4)
C(21)	2,235(2)	5,328(2)	9,758(3)	571(8)	−1,278(2)	802(2)	7,613(3)	539(5)
O(21)	1,419(2)	5,448(3)	10,655(3)	800(9)	−2,583(2)	825(2)	7,703(3)	692(6)
O(20)	1,954(2)	7,038(2)	9,390(2)	678(7)	−2,066(1)	1,525(2)	4,634(2)	712(6)
O(3)	10,898(2)	5,326(2)	7,052(3)	856(9)	9,093(1)	3,944(2)	10,226(3)	725(6)

* B_{eq} is calculated as $B_{eq} = 4/3 \sum_{ij} \beta_{ij} a_i \cdot a_j$.

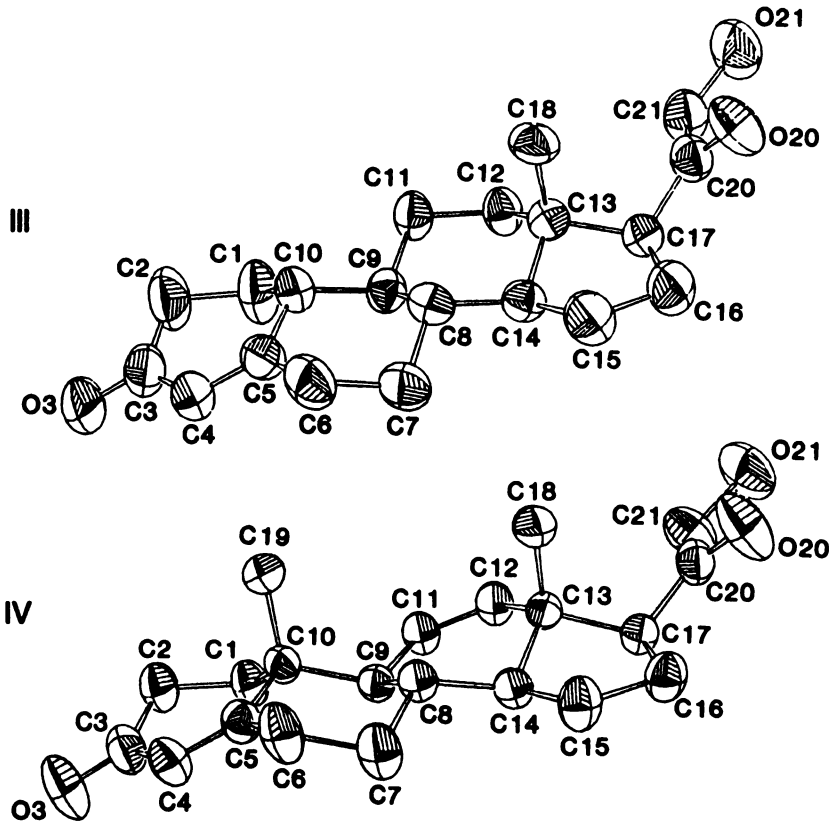

Fig. 1. Atomic numbering and observed conformations of 19-nor-11-deoxycorticosterone (**III**) and 21-hydroxypregna-4,11-diene-3,20-dione (**IV**). Thermal ellipsoids are shown at the 80% probability level.

value of **I** was the lowest. This is the least flat compound because three substituent groups, the 19-methyl, 18-methyl, and 11β-hydroxy groups, are in the 1,3-diaxial positions to each other, and steric repulsions exist between 11β-hydroxy and 19-methyl or 18-methyl groups. This repulsion causes bowing of the molecular structure to the α side. Removal of 11β-hydroxy and/or 19-methyl groups leads to flatter molecules, **II** and **III**, by diminishing the steric repulsion between the substituents.

TABLE 3
RBAs of aldosterone derivatives and their physical properties

No.	log RBA[a]	DO[b]	R_m[c]	log P[d]	^{13}C-Chemical shift (ppm)[e]					Stretching band (cm^{-1})[f]		
					δ(20)	δ(3)	δ(4)	δ(5)	δ(21)	C(3)═C(4)	C(3)═O	C(20)═O
I	1.15	2.011	0.227	1.26	210.0	199.5	122.4	172.0	69.2	1618	1664	1708
Va	1.70	1.945	0.046	0.67	209.8	199.4	124.3	170.2	69.1	1615	1666	1713
Vb		2.014										
II	1.76	1.878	0.445	3.35	210.1	199.3	124.0	170.6	69.4	1616	1663	1707
VIa	2.00	1.448	0.112	0.32	107.0	199.3	124.5	169.8	68.8	1616	1667	
VIb				−0.14	209.9	199.3	124.4	169.8	68.8	1616	1667	1704
IV	2.18	1.155	0.448	2.80	210.0	199.1	125.2	169.2	69.3	1615	1663	1708
III	2.23	1.021	0.366	2.83	210.1	199.7	124.8	166.0	69.4	1619	1664	1706

[a] RBA = 100 × $^{50}_{0}$C/^{50}C, where $^{50}_{0}$C and ^{50}C are concentrations of aldosterone and the competitor, respectively, at 50% inhibition of ^{3}H-aldosterone bindings of control. RBA values are cited from Ref. 5.
[b] The distance of the oxygen atom in the A ring from the least squares plane through atoms C(5) to C(14), measured in Å.
[c] The interpolated value at 50% acetone, which was evaluated by an r-TLC method (11).
[d] Estimated by the CLOGP-3 program (12, 13).
[e] In $CDCl_3$. Tetramethylsilane was used as an internal standard.
[f] Infrared spectra were measured in $CHCl_3$ solution.

Compound **III** would be flatter than **II** because it has no substituent at positions 10 and 11. The RBA order actually found was **III** > **II** > **I**. Of the six steroids, **III** has the highest RBA and is obviously the flattest as shown by our X-ray crystallographic analysis. We considered the possibility of the highest RBA of **III** resulting from the diminishing of the steric effect of the 19-methyl group due to a compound fitting in the active site of the receptor. However, in our case, this possibility seemed to be small because the RBA of **III** did not notably deviate from Eq. 2, described later, which expresses the relationship of the RBA to the flatness of a molecule.

Although introduction of a double bond to position 11 generally increases the planarity of the molecular structure, the steric repulsion between the 18- and 19-methyl groups makes the structure bow to the α side. As a result, the planarity of **IV** becomes higher than that of **II** and lower than that of **III**. Thus, its RBA value is just between those of **II** and **III**. The structures of **V** are flatter than that of **I** due to elimination of 1,3-diaxial repulsion between 11β- and 18-substituents by 11β,18-epoxide formation although the repulsion between 11β- and 19-substituents is not removed. This would be the reason that its RBA is larger than that of **I**. Crystals of this compound have been reported to contain two crystallographically independent molecules, **Va** and **Vb**, as shown in Fig. 2 (1). We presumed the former would be an active form, according to the suggestion of Duax *et al.* (1) that molecules with the conformation of **Va** compete with aldosterone **(VI)** for receptor binding due to the similarity of its A ring conformation and hydrogen bonding in the 17β-substituent to that of **VI** which is a natural mineralocorticoid hormone. Compound **VI** has been postulated to be in an equilibrium among three structural isomers: the 18-acetal-20-hemiketal **(VIa)**, the 11β,18-oxide **(VIb)**, and the 18-aldehyde **(VIc)** (14). In solution, the existence of isomers **VIa** and **VIb** has been spectroscopically confirmed (15). In contrast, in crystals, **VI** has only the **VIa** structure (8). The higher RBA of **VI** can be explained more by form **VIa**, which is as planar as **III** with the highest RBA as shown in Fig. 2, rather than by other forms, which would not be good planar forms: the molecular structure of **VIb** and **VIc** would be similar to that of **V** and **I**, respectively, as supposed from their molecular forms.

We attempted to express quantitatively the structure-activity relationships described qualitatively above and found good relationships of the RBA with the distance of the oxygen atom (*DO*) in the A ring from the least squares plane through atoms C(5) to C(14) of the compounds. The relationship is given by Eq. 2:

$$\log \text{RBA} = -0.823DO + 3.13$$
$$(n = 6,\ r = -0.885,\ s = 0.207) \quad (2)$$

This equation shows that RBA increases with decreasing *DO* value, that is, with increasing planarity of the molecular structure, and confirms that the flatness of a molecule plays an important role in its binding to the receptor.

Electronic effects. The change of the electronic structure of the C(5) atom may have some influence on RBA. The log RBA has a good relationship with the ^{13}C-NMR chemical shifts, δ(5), of the C(5) atom. The correlation is given by the following equation:

$$\log \text{RBA} = -0.164\delta(5) + 29.7$$
$$(n = 6,\ r = -0.829,\ s = 0.250) \quad (3)$$

The chemical shift of a carbon atom is correlated to the electron density on it (16); an increase of the latter causes a decrease of the former. Therefore, Eq. 3 suggests that an increase of the electron density on the carbon at position 5 leads to an increase of log RBA. It should be noted that a change in the electronic structure at the C(5) atom would result from the conformational change in the molecule.

All of the compounds discussed seem to have similar electronic structures for the carbonyl group in the A ring. This is supported by the fact that, in chloroform solution, the respective values for the stretching band positions of the carbonyl group in the A ring and their ^{13}C chemical shift values for C(3) were almost the same, as shown in Table 3. Also, the spectroscopic data in Table 3 suggest that the carbonyl group in the 17β-side chain has the same electronic structure in all of the steroids except for **VIa**, which has no such group in the side chain. Therefore, the steroids discussed seem to display the same electronic interaction with the MR at the carbonyl group in the A ring and also at that in the 17β-side chain. Conversely, the change in the electronic structure in the C(5) atom induced by the conformational change may make some contributions to the binding for the receptor through weak electrostatic interaction, which would aid the major interaction of the carbonyl

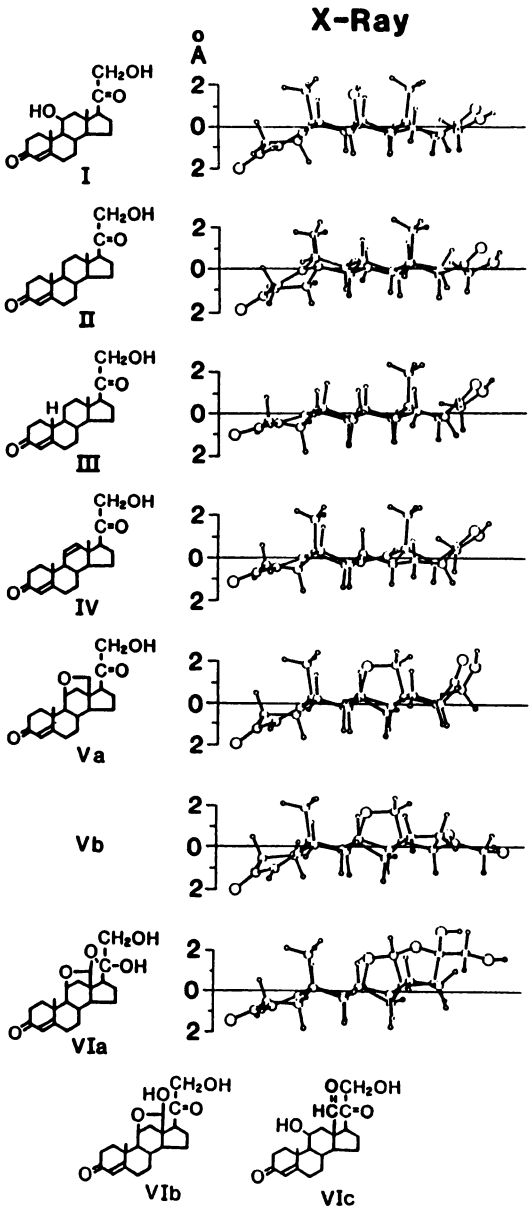

Fig. 2. Conformations of aldosterone derivatives obtained by X-ray crystallographic analysis.

group in the A ring with the binding site of the receptor. However, the contributions seem to be relatively small and thus minor because the differences in $\delta(5)$ are relatively small in comparison with those in the conformation of the A ring: the differences (6 ppm) in $\delta(5)$ correspond to those of 0.02 electron according to Henry and Fliszár (16).[2]

Hydrophobic effects. Few relationships were found between the RBA value and the R_m value or the log P value calculated by the CLOGP-3 program as shown in Table 3 (12, 13). The hydrophobic interaction does not seem to be a dominant factor in the binding to the MR, although such an interaction is very important in the binding to the glucocorticoid and the progestin receptors (17, 18). Therefore, the binding site in the MR does not seem to be hydrophobic, nor does a steroid compound appear to approach the site through the hydrophobic environment in the receptor.

[2] Although we analyzed our data with a multiple regression method in which more than two independent variables (*DO*, $\delta(5)$, and log P or R_m) were used, we could obtain no statistically significant result, probably because the number of observations was insufficient: six data points are too few for multiple regression analysis of more than two independent variables. However, six data points were a sufficient number of observations for simple regression analysis.

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