Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 757–760. Pergamon Press. Printed in Great Britain

POSSIBLE HYDROGEN-BOND INTERACTIONS OF STEROID HORMONES—II. CORTICOSTEROIDS*

O. DIDEBERG, L. DUPONT and H. CAMPSTEYN

Laboratoire de Cristallographie, Institut de Physique, Université de Liège au Sart Tilman, B-4000 Liège, Belgium

(*Received* 9 *October* 1975)

SUMMARY

For each of the polar functions of the twenty known crystalline structures of corticosteroids, we determine the position of the atoms forming an intermolecular hydrogen bond with the oxygen of the function, so that we can find the preferential positions—relative to the steroid—of the atoms that take part in such bonds. That leads us to an estimate of the distance between the atoms capable of fixing the steroid by O_3 and O_{20} or O_{21} on the receptor. The determined value is 16.5 Å. We apply the same reasoning to explain the role of the inhibitors.

INTRODUCTION

The crystal structure data of twenty corticoids have been published [1–20], their names are given in Table I. These accurate X-ray determinations of the molecular structures of steroids provide a basis upon which it is possible to build a quantitative analysis of the conformational patterns.

Work published since 1950 on the biological activity of many synthetic or natural molecules has made it possible to bring out a systematic relation between the substituents and the biological activity [21]. The determination of the crystalline structures of the corticosteroids indicates variations of conformation of the steroid generated by the different substitutions. Attempts at explanations of the correlations between molecular structure and function have been made with the help of these results [22–23]. Starting from these crystalline structures, we can also determine the positions occupied by the atoms forming intermolecular hydrogen bonds with the polar functions of the corticosteroids. This question is of capital importance, for some of these are essential to any biological activity.

* This paper was presented at the Round Table: Réunion annuelle de l'Association Française de Cristallographie—Liège Mai 1975.

METHODOLOGY

We have examined the results of X-ray analysis of 20 corticosteroids (Table 1). For each function C=O

Table 1. Corticosteroids used in this publication*

1.	9α-Fluorocortisol
2.	9α-Chlorocortisol
3.	9α-Bromocortisol
4.	Cortisol (pyridine solvate (1:1))
5.	Cortisol (.methanol solvate (1:1))
6.	Cortisone
7.	21β-acetoxy-17-hydroxy-4-pregnene-3,11,20-trione (Cortisone acetate)
8.	11-Deoxycortisol
9.	Corticosterone
10.	Progesterone (.Resorcinol complex (1:1))
11.	17α-Hydroxyprogesterone
12.	4-Chlorocortisone
13.	12α-Bromo-11β-Hydroxyprogesterone
14.	9α-Fluoro-11β,21-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione (17-Desoxymethasone)
15.	11β, 16α, 17, 21-Tetrahydroxy-1,4-pregnadiene-3,20-dione (16α-Hydroxyprednisolone)
16a.	9α-Fluoro-6α-methyl-11β,17, 21-trihydroxy-1,4-pregnadiene-3,20-dione. A. (9α-Fluoro-6α-methyl prednisolone)
16b.	9α-Fluoro-6α-methyl-11β,17, 21-trihydroxy-1,4-pregnadiene-3,20-dione B.
17.	6α-Methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione (6α-Methyl prednisolone)
18.	6α-Fluorocortisol
19.	20(S)-Hydroxy-4-pregnene-3-one
20.	21-Acetoxy-9α-fluoro-16α-methyl-11β,17-dihydroxy-1,4-pregnadiene-3,20-dione (Dexamethasone Acetate)

* In this table, the number corresponds to the numbering of the compound in Fig. (1–5) and of the references.

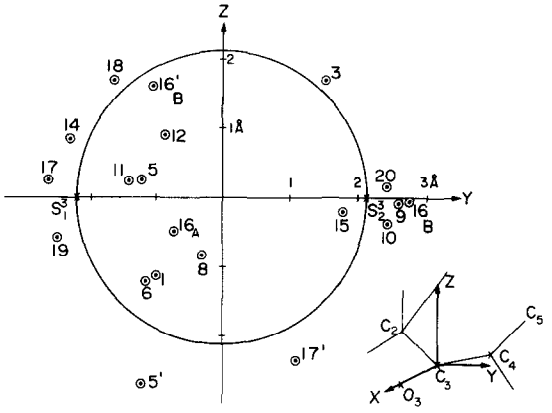

Fig. 1. Projection along C_3—O_3. The frame of reference is defined by X along C_3—O_3, Y in the plane (C_3, O_3, C_4) and Z perpendicular to X and Y.

and C—O—H, we define a frame of reference (Fig. 1–5) and in this we place the donor or acceptor atom of the neighbouring molecules (called S atom). We have selected the atoms involved in a H bond whose length is less than 3 Å. In the figures, the number (1–20) corresponds simultaneously to the numbering of the compound in Table 1 and references. If the same polar function binds two S atoms, the second number is prime. In every case, we calculate $\bar{d}$, mean distance O...S and $\bar{\Omega}$ mean angle C—O...S. These two results allow us to describe a circle on which the S atom lies in a preferential way. Figure 6 shows a specific example for $C_3 = O_3$. Along this circle some positions are not allowed, the contact between S and the steroid atoms, measured by Δ, being too short. So we define 3 regions: $\Delta < 3$ Å "forbidden" region (double line ═), 3 Å $\leqslant \Delta \leqslant$ 3.4 Å "tolerated" region (broken line, – – –) and $\Delta > 3.4$ Å "allowed" region (continuous line, ——). On the other hand, we can observe areas with high concentrations of experimental points; so it is possible to define the preferential positions of the donor or acceptor sites, called S_j^i, with $i = 3$ for O_3, $i = 11$ for O_{11}, ... and $j = 1$, n. (n = number of sites for this polar function, cf. Fig. 1–5).

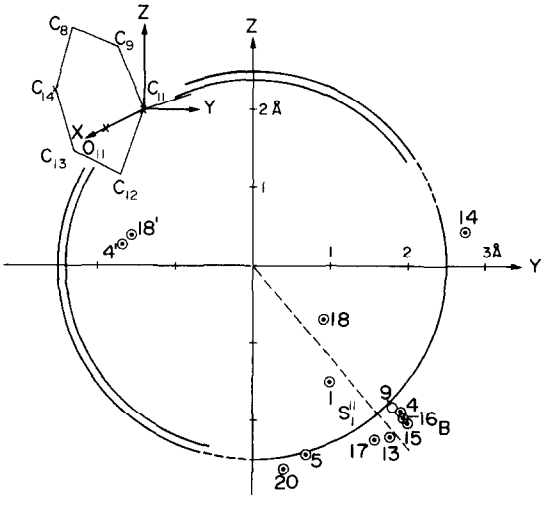

Fig. 2. Projection along C_{11}—O_{11}. The frame of reference is defined by X along C_{11}—O_{11}, Y in the plane (C_{11}, O_{11}, C_{14}) and Z perpendicular to X and Y.

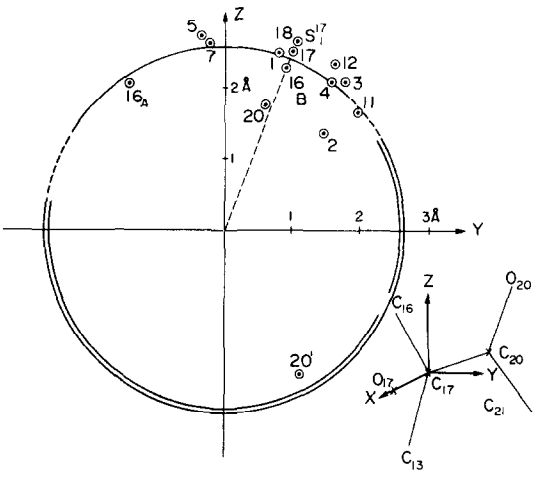

Fig. 3. Projection along C_{17}—O_{17}. The frame of reference is defined by X along C_{17}—O_{17}, Y in the plane (C_{17}, O_{17}, C_{20}) and Z perpendicular to X and Y.

RESULTS

In Table 2, we have recorded the mean O...S and C—O...S values.

The S_j^i sites being known, we can replace them in a frame of reference relative to a mean corticosteroid [24]. This gives Fig. 7 which is a stereoscopic view of the "mean corticosteroid" molecule with S_j^i positions.

Table 3 shows the value of the distances between these sites.

DISCUSSION

(a) C_3═O_3:(Fig. 1). The points are distributed on either side of the plane Z = 0 and form two clusters

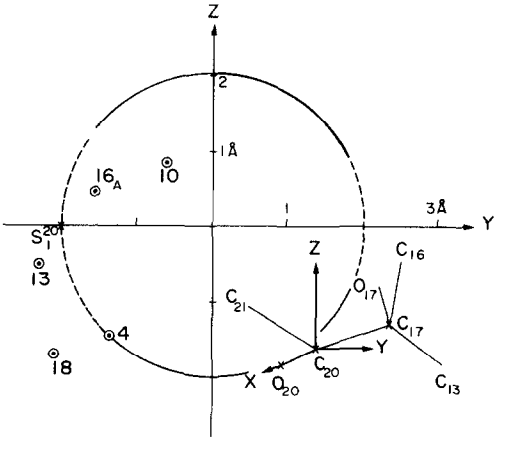

Fig. 4. Projection along C_{20}—O_{20}. The frame of reference is defined by X along C_{20}—O_{20}. Y in the plane (C_{20}, O_{20}, C_{17}) and Z perpendicular to X and Y.

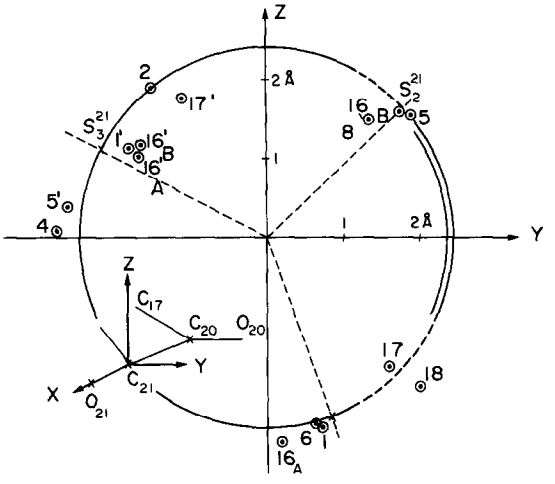

Fig. 5. Projection along C_{21}—O_{21}. The frame of reference is defined by X along C_{21}—O_{21}. Y in the plane (C_{21}, O_{21}, C_{20}) and Z perpendiccular to X and Y.

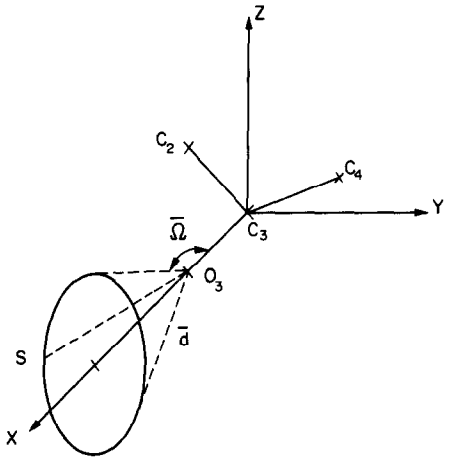

Fig. 6. Représentation of geometrical parameters $\bar{d}$ and $\bar{\Omega}$

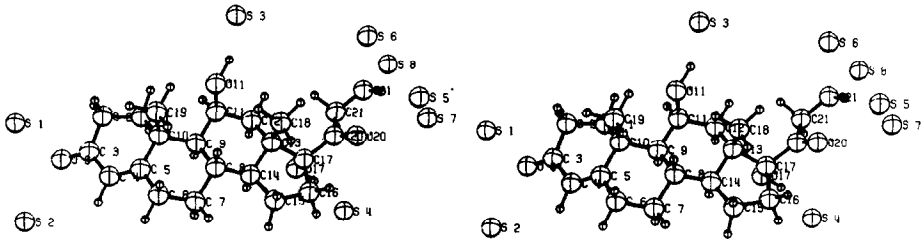

Fig. 7. Stereo-view of the corticosteroid molecule and sites $S_1 = S_1^3$, $S_2 = S_2^3$, $S_3 = S_1^{11}$, $S_4 = S_1^{17}$, $S_5 = S_1^{20}$, $S_6 = S_1^{21}$, $S_7 = S_2^{21}$, $S_8 = S_2^{21}$.

whose centers lie approx. in that plane. So we can define two sites S_1^3, S_2^3 in this plane. Computation of the distances between donor atoms and their neighbours shows that the "allowed" region includes the whole circumference. As one might expect, this function is accessible.

(b) C_{11}—$O_{11}H$:(Fig. 2). The "allowed" region is more limited and the experimental points form one group. The points [4' 18'] localized in the "forbidden" region are associated with the two other ones situated in the "allowed" region. In both cases the C—O...S angle is wide open (141° and 147°).

(c) C_{17}—$O_{17}H$:(Fig. 3). The points are concentrated on one side of the "allowed" region. Though this area is rather limited, it should be noted that O_{17} is involved in many H bonds.

(d) C_{20}=O_{20}:(Fig. 4). The function C_{20}=O_{20} takes part in very few H bonds. The points in the plane $Z = 0$ are situated in a "tolerated" region, which explains the weak capacity of O_{20} in forming H bonds. This finding is important, because O_{20} is often considered as essential to any biological activity.

(e) C_{21}—$O_{21}H$:(Fig. 5). The "forbidden" region is relatively limited. The points are found to gather in

Table 2. Observed mean bond lengths (Å) and mean angles (°) (arithmetic mean) n is the number of values in the sample

	$\bar{d}$ (Å)	$\bar{\Omega}$ (°)	n
O_3	2.845	131.3	20
O_{11}	2.883	119.3	13
O_{17}	2.842	110.9	14
O_{20}	2.845	135.5	5
O_{21}	2.812	119.9	15

Table 3. Some distances between the sites (Å)

	S_1^3	S_2^3	S_1^{11}	S_1^{17}	S_1^{20}	S_1^{21}	S_2^{21}	S_3^{21}
S_1^3		4.26	10.07	13.95	16.41	14.73	16.70	15.65
S_2^3			11.63	13.13	16.32	15.37	16.61	16.43
S_1^{11}				9.27	7.87	5.25	8.65	7.51
S_1^{17}					6.42	7.63	5.30	6.33
S_1^{20}						3.10	2.04	4.98
S_1^{21}							4.22	4.51
S_2^{21}								3.87
S_3^{21}								

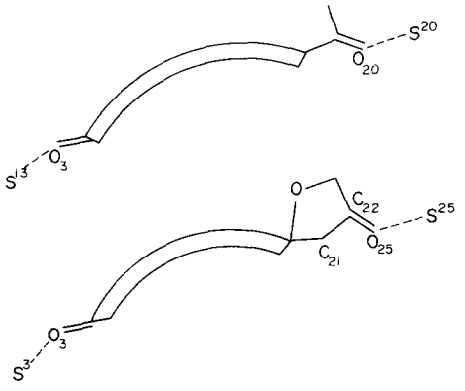

Fig. 8. Schematic representations for Progesterone and Spironolactone.

3 zones. The lower left quadrant is unoccupied. This may result from the fact that the H bonds we have studied generally bind two steroids. Another explanation would consist in considering the Y direction as the most favourable to the orientation of O_{21}...S. This being unfavourable from a steric point of view, O_{21}... S would lie in a preferential way towards S_2^{21} or S_1^{21}; in both cases the second lone pair is in the direction of S_3^{21}.

Although they are means of a small sample, the distances O....S of Table 2 show an evolution in the interaction forces $O_{21} > O_{17}$, O_3, $O_{20} > O_{11}$.

For all these calculations, the molecules are supposed to be undistorted by the crystalline field. We shall assume that the same holds true in the solved state.

Table 3 calls for some remarks: (a) the distances S_i^3—S_1^{20} are about 16.4 Å. Progesterone and 7α-acetylthio-3-oxo-17α-4-pregnene-21, 17β carbolactone (spironolactone) are inhibitors by competition with the mineralocorticoids [25, Table 1]. We wanted to compare these two molecules. The polar functions capable of playing the same role in binding with the receptor are C_3=O_3 for both molecules, C_{20}=O_{20} of progesterone and C_{22}=O_{25} [26] for spironolactone (Fig. 8). By taking the parameters of Table 2, we can calculate the positions S_j^3 connected with the spironolactone, as well as those forming a H bond with O_{25}, (called S^{25}). The calculation of the distances S_j^3—S_j^{25} shows that for a torsional angle C_{21}—C_{22}—O_{25}—S_j^{25} of 150° we obtain 16.4 Å, the orientation of both steroids being nearly the same. This could perhaps explain the affinity of the spironolactone for the mineralocorticoid receptor. We shall also emphasize, that cortisone (inhibitor) and 9α fluoro cortisol (very active) are isostructural (same unit cell and same packing).

Biological tests [27, Table 23] have shown that the activity is increased by the hydroxyl substitution in C_{21}. This could be explained by the proximity of O_{21} and S_1^{20} (d = 3.14 Å), which would strengthen the steroid receptor bond.

A more likely possibility would consist in having a critical distance of about 16.50 Å for the fixation on the mineralocorticoid receptor. This has been realized by S_i^3—S_1^{20} in the case of corticosteroid without hydroxyl in 21 positions and by S_i^3—S_2^{21} in the case of corticosteroid with hydroxyl 21.

Acknowledgements—The authors thank Professors Toussaint and Brasseur for the interest they have taken in this work.

REFERENCES

1. Dupont L., Dideberg O. and Campsteyn H.: *Acta crystallogr.* **B28** (1972) 3023–3032.
2. Weeks C. M., Duax W. L. and Wolff M. E.: *Acta crystallogr.* **B30** (1974) 2516–2519.
3. Weeks C. M. and Duax W. L.: *Acta crystallogr.* **B29** (1973) 2210–2213.
4. Campsteyn H., Dupont L. and Dideberg O.: *Acta crystallogr.* **B30** (1974) 90–94.
5. Roberts P. J., Coppola J. C., Issacs N. W. and Kennard O.: *JCS Perkin* **II** (1972) 774–780.
6. Declercq J. P., Germain G. and van Meerssche M.: *Cryst. struct. Commun.* **1** (1972) 13–15.
7. Declercq J. R., Germain G. and van Meerssche M.: *Cryst. struct. Commun.* **1** (1972) 59–61.
8. Dupont L., Dideberg O. and Campsteyn H.: *Acta crystallogr.* **B29** (1973) 205–214.
9. Campsteyn H., Dupont L., Dideberg O. and Mandel N.: *Acta crystallogr* **B29** (1973) 1726–1728.
10. Dideberg O., Dupont L. and Campsteyn H.: *Acta crystallogr.* **B31** (1975) 637–640.
11. Declercq J. P., Germain G. and van Meerssche M.: *Cryst. struct. Commun.* **1** (1972) 9–11.
12. Duax W. L., Cooper A. and Norton D. A.: *Acta crystallogr.* **B27** (1971) 1–6.
13. Cooper A. and Norton D. A.: *Acta crystallogr* **B24** (1968) 811–823.
14. Dupont L., Dideberg O. and Campsteyn H.: *Acta crystallogr.* **B30** (1974) 514–516.
15. Dideberg O., Dupont L. and Campsteyn H.: *Acta crystallogr.* **B30** (1974) 2064–2066.
16. Dideberg O., Dupont L. and Campsteyn H.: *Acta crystallogr.* **B30** (1974) 702–710.
17. Declercq J. P., Germain G. and van Meerssche M.: *Cryst. struct. Commun.* **1**, (1972) 5–7.
18. Duax W. L.: Private communication. Cryst. Structure of 6α F cortisol (1972).
19. Isaacs N. W., Motherwell W. D. S., Coppola J. C. and Kennard O.: *JCS Perkin* **II** (1972) 2331–2335.
20. Terzis A. and Theuphanides R.: *Acta crystallogr* **B31** (1975) 796–801.
21. Bush J. E.: *Pharmac. Rev.* **14** (1962) 317–419.
22. Weeks C. M., Duax W. L. and Wolff M. E.: *J. Am. chem. Soc.* **95** (1973) 2865–2868.
23. Dideberg O., Dupont L. and Campsteyn H.: Communication at the Stockholm Symposium on the Structure of Biological Molecules July 9–12 (1973).
24. Campsteyn H., Dideberg O. and Dupont L.: (to be published).
25. Albert K. G. M. M. and Sharp G. W. G.: *J. Endocr.* **48** (1970) 563–574.
26. Dideberg O. and Dupont L.: *Acta crystallogr.* **B28** (1972) 3014–3022.
27. Fried J. and Borman A.: *Vitam. Horm.* **16** (1958) 303–374.