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# CORRELATION OF GLUCOCORTICOID AND PROGESTATIONAL ACTIVITY WITH STERIC, ELECTRONIC AND HYDROPHOBIC PARAMETERS

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## SUMMARY

In this work an attempt is made to explain the effect of activity enhancing groups in steroids in terms of their physico-chemical effects on the parent molecule. The following equation was derived to correlate the activities of 9α-substituted cortisol derivatives: Log $A = 0{\cdot}07 + 0{\cdot}76\pi - 0{\cdot}22 \log P_E + 2{\cdot}786^I$. We found through X-ray crystallographic studies and CNDO/2 calculations that the conformation and electron distribution of the 9α-substituted cortisol derivatives is markedly affected by the nature of the 9α-substituent. These results were correlated with the extent of binding of the steroid to the rat hepatoma cell glucocorticoid receptor. In related work, the progestational activity of 6-substituted-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20 diacetate derivatives was correlated equally well by the equations: Log $A = 0{\cdot}17 + 1{\cdot}06\pi = 3{\cdot}46F - 10{\cdot}5\pi^2$ or Log $A = 0{\cdot}26 - 0{\cdot}07MR + 0{\cdot}97\pi + 2{\cdot}84F$.

## INTRODUCTION

I would like to present here some of our results in the correlation of steroid structure with biological activity. In doing this I would like to remind you of something that you already know very well, and that is that we are trying to fit a physical and/or mathematical model to an exceedingly complex system. One of the principles of such modeling is that it is possible to construct a complete, relatively complex model for a simple system, but when we are dealing with a complex system such as this one, we must be satisfied with a simple model.

What I would like to do is to show you some of the approaches we have made using two separate steroid systems and to discuss some of the problems we have encountered. I want to emphasize that these studies represent only approaches to the question of the correlation of activity with structure, and certainly do not give any final answers. Yet I believe that the quantitative prediction of the effects of structural change on biological activity is a central problem today in medicinal chemistry. And I believe also that, because steroids are relatively rigid molecules where the effects of structural change are easily understood in steric terms, and because we know something of the mechanism of action of steroids, that these molecules are one of our best hopes for studies in quantitative structure activity relationships.

## DISCUSSION

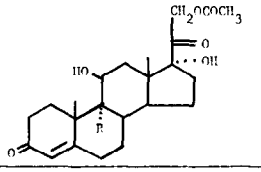
A given molecular modification in a steroid could exert its effect through a variety of steric, inductive or hydrophobic bonding mechanisms. Such complex relationships are common to structure-function analyses in many drugs, and the separation of some of these variables has been greatly facilitated by the multiple parameter approach [1]. We now describe the application of this approach to steroids for the first time. In this report we consider the anti-inflammatory activity [2a–d] of 9α-substituted cortisol derivatives (Table 1). Previous workers [2b] attempted to account for the biological influence of 9α-substituents on the basis of their inductive effects, but this factor explains only a portion of the variation (cf. Equation 3).

Rather than attempting to derive *de novo* constants [3a–d], we utilized the stochastic method using known physicochemical parameters [1] for the inductive effect ($\sigma_I$) [4], the hydrophobic bonding power ($\pi$) [5], and the size of substituents (molar refractivity, $P_E$) [6] and ($E_S$) [7]. From the data in Table 1 we derived *via* the method of least squares Equations (1–7). In these equations, $n$ represents the number of data points used in the regression, $r$ is the correlation coefficient and $s$ is the standard deviation.

Of the single-parameter Equations (1–3), the one with $\pi$ gives the poorest result. Independently $\sigma_I$ and

| | $n$ | $s$ | $r^2$ | $r$ |
|---|---|---|---|---|
| (1) log $A = -0{\cdot}1799 - 0{\cdot}2248\pi$ | 7 | 0·8708 | 0·0378 | 0·1943 |
| (2) log $A = 0{\cdot}3219 - 0{\cdot}0959 \log P_E$ | 7 | 0·7433 | 0·2989 | 0·5467 |
| (3) log $A = -0{\cdot}7408 + 1{\cdot}7707\sigma_1$ | 7 | 0·7791 | 0·2296 | 0·4792 |
| (4) log $A = 0{\cdot}5992 + 0{\cdot}5821\pi - 0{\cdot}1623 \log P_E$ | 7 | 0·7634 | 0·4084 | 0·6391 |
| (5) log $A = -0{\cdot}7256 - 0{\cdot}2965\pi + 1{\cdot}8857\sigma_1$ | 7 | 0·8337 | 0·2943 | 0·5425 |
| (6) log $A = -0{\cdot}2385 - 0{\cdot}1298 \log P_E + 2{\cdot}5396\sigma_1$ | 7 | 0·5120 | 0·7339 | 0·8567 |
| (7) log $A = 0{\cdot}0726 + 0{\cdot}7642\pi - 0{\cdot}2202 \log P_E + 2{\cdot}7794\sigma_1$ | 7 | 0·3267 | 0·9187 | 0·9585 |

Table 1. Liver glycogen deposition activity and substituent constants for 9α-substituted cortisol derivatives



| R | Obsd. Rel. Act. [a] | Calcd. Rel. Act. | Obsd. log A | Calcd. log A [b] | $\sigma_I$ [c] | $\pi$ [d] | Log $F_S$ [e] | Log $P_E$ [f] |
|---|---|---|---|---|---|---|---|---|
| F | 10.7 | 13.3 | 1.03 | 1.124 | 0.52 | -0.17 | 0.78 | 1.20 |
| Cl | 4.7 | 2.3 | 0.67 | 0.364 | 0.47 | 0.39 | 0.27 | 5.96 |
| Br | 0.3 | 0.7 | -0.52 | -0.169 | 0.45 | 0.60 | 0.08 | 8.86 |
| I | 0.1 | 0.1 | -1.00 | -1.168 | 0.38 | 1.00 | -0.16 | 13.90 |
| OH | 0.2 | 0.2 | -0.70 | -0.696 | 0.25 | -1.16 | 0.69 | 2.62 |
| H | 1.0 | 0.7 | 0.00 | -0.170 | 0.0 | 0.0 | 1.24 | 1.10 |
| $CH_3$ | 0.1 | 0.2 | -1.00 | -0.805 | 0.0 | 0.50 | 0.0 | 5.72 |
| OMe | 0.0 [g] | 0.1 | - | -1.186 | 0.25 | -0.47 | 0.69 | 7.24 |
| OEt | 0.0 [g] | 0.0 | - | -1.821 | 0.25 | 0.03 | 0.69 | 11.86 |
| SCN | 0.0 | 0.0 | - | -1.864 | 0.55 | 0.03 | 0.17 | 15.84 |

[a] Relative activity (cortisol acetate = 1) from ref. 2. [b] Calcd. using Equation (7). [c] From ref. 4. [d] From ref. 5. [e] From ref. 7. [f] From ref. 6. [g] This compound was resynthesized and the biological activity remeasured by the same technique by Endocrine Laboratories, Madison, Wisconsin. We thank Dr. Winston Ho for the synthetic work.

$P_E$ account for only 23% and 30%, respectively, of the variance in the data. Together they account for 73% of the variance. In the two-parameter equations, little, if anything, is gained using $\pi$.

Equation (7) gives the best correlation. This equation satisfies the "*F*-test" [8] and is statistically significant at the 0·95 confidence level, $F_{3,3} = 11{\cdot}3$ ($F_{3,3}$ at the 0·95 level is 9·6). Equation (7) is a significant improvement over the two parameter Equation (6); ($F_{1,3} = 6{\cdot}8$, $F_{1,3\ \alpha=0{\cdot}1} = 5{\cdot}5$). Ideally, one would like to have 15 data points for a three variable equation. In effect, there are 10 data points in the present case, but only 7 have been used in deriving the equation. Although inactive compounds cannot be used to fit the regression, nevertheless, the fact that Equation (7) predicts the SCN, OET and OMe compounds to have little or no activity gives confidence in its correctness.

The positive coefficient with $\sigma_I$ and with $\pi$ indicates activity is promoted by electron-withdrawing groups and by groups of high hydrophobicity. The negative coefficient with $P_E$ indicates an increasing activity with decreasing size of the substituent.

Recently, we found through X-ray crystallographic studies [9] and CNDO/2 calculations [10] that the conformation and electron density distribution of the 9α-substituted cortisol derivatives is markedly affected by the nature of the 9α-substituent. Major changes are seen in the conformation of the A-ring, and in the electron density on the 11β-OH and at C-4. These effects offer an explanation for the high dependence on steric and electronic terms in Equation (7). Clearly, the inductive nature of the 9α-substituent will affect its influence on the electron density distribution in the substituted compound, whereas the steric influence will have conformational and electronic consequences. The use of Equation (7) to evaluate the biological effect of a substituent is, of course, far easier and more direct than the CNDO/2 approach.

The significance of the $\pi$ parameter is more difficult to delineate. The increase in activity with increasing hydrophobicity could be due to better transport to the site of action for more lipophilic compounds and/or to hydrophobic interaction at the active site [11]. The fact that large groups (as measured by $P_E$) are only weakly active indicates that α-substituents may have to fit the receptor site, a situation in harmony with a positive role for $\pi$ for 9α-functions. Although it has been suggested that corticoids interact with the receptor on the β-face [12], this result could be evidence that the 9α-substituent does in fact interact with the receptor.

Equation (7) offers the chance to predict more active corticoids but few substituents meet the requirements. A 9α-$CF_3$ group should have activity of about 4·0 relative to hydrocortisone, but most groups with the required hydrophobicity are too large to be active.

Recently Teutsch *et al.*[13] attempted to relate the steric and electronic characteristics of C(6)-substituents in 6-substituted-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate derivatives to the effect of such substituents on progestational (Clauberg) activity. These authors derived steric indexes based on bond lengths and van der Waals radii and used broad estimates of electronic features to reach their conclusions. Their treatment was qualitative and did not consider the partition coefficient of the molecules. In the present report we describe the application of the multiparameter method to the foregoing progesterone derivatives (Table 2) and the obtainment of a quantitative relationship differing from the conclusions drawn by Teutsch *et al.*[13].

| | $n$ | $s$ | $r^2$ | $r$ |
|---|---|---|---|---|
| (1) $\log A = 1{\cdot}45\ (\pm 1{\cdot}15) - 0{\cdot}09\ (\pm 0{\cdot}12)\ MR$ | 13 | 0·875 | 0·198 | 0·444 |
| (2) $\log A = 0{\cdot}60\ (\pm 0{\cdot}40) + 1{\cdot}14\ (\pm 0{\cdot}66)\ \pi$ | 13 | 0·645 | 0·564 | 0·751 |
| (3) $\log A = -0{\cdot}29\ (\pm 1{\cdot}55) + 2{\cdot}94\ (\pm 4{\cdot}39)\ F$ | 13 | 0·892 | 0·165 | 0·406 |
| (4) $\log A = -0{\cdot}41\ (\pm 1{\cdot}74) + 3{\cdot}15\ (\pm 4{\cdot}72)\ F - 0{\cdot}52\ (\pm 2{\cdot}54)\ R$ | 13 | 0·926 | 0·182 | 0·427 |
| (5) $\log A = -1{\cdot}11\ (\pm 0{\cdot}84) - 0{\cdot}06\ (\pm 0{\cdot}09)\ MR + 1{\cdot}04\ (\pm 0{\cdot}65)\ \pi$ | 13 | 0·611 | 0·644 | 0·803 |
| (6) $\log A = 0{\cdot}43\ (\pm 1{\cdot}61) - 0{\cdot}10\ (\pm 0{\cdot}11)\ MR + 3{\cdot}31\ (\pm 3{\cdot}95)\ F$ | 13 | 0·790 | 0·405 | 0·636 |
| (7) $\log A = -0{\cdot}24\ (\pm 1{\cdot}01) + 1{\cdot}10\ (\pm 0{\cdot}60)\ \pi + 2{\cdot}54\ (\pm 2{\cdot}86)\ F$ | 13 | 0·574 | 0·686 | 0·828 |
| (8) $\log A = 0{\cdot}79\ (\pm 0{\cdot}63) + 1{\cdot}13\ (\pm 0{\cdot}68)\ \pi - 0{\cdot}53\ (\pm 1{\cdot}35)\ \pi^2$ | 13 | 0·652 | 0·595 | 0·771 |
| (9) $\log A = 0{\cdot}26\ (\pm 1{\cdot}01) - 0{\cdot}07\ (\pm 0{\cdot}07)\ MR + 0{\cdot}97\ (\pm 0{\cdot}53)\ \pi + 2{\cdot}84\ (\pm 2{\cdot}50)\ F$ | 13 | 0·489 | 0·795 | 0·892 |
| (10) $\log A = -0{\cdot}17\ (\pm 0{\cdot}88) + 1{\cdot}06\ (\pm 0{\cdot}52)\ \pi + 3{\cdot}46\ (\pm 2{\cdot}68)\ F - 1{\cdot}05\ (\pm 1{\cdot}11)\ \pi^2$ | 13 | 0·492 | 0·792 | 0·890 |

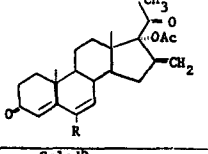
From the data in Table 2 we derived Equations (1–10) by the method of least squares. The three parameter Equations (9 and 10) both give high correlations. Both equations satisfy the $F$-test [8] at the 0·005 level, having $F$ values of 11·6 and 11·4 respectively. In these equations, the $\pi^2$ and $MR$ (steric) terms have a similar effect, and it is not possible to say which equation represents the data more accurately with the information at hand. The use of all four parameters does not improve the situation. Both equations indicate that activity is promoted by electron-withdrawing groups and by lipophilic groups. However, Equation (10) predicts that activity reaches a maximum with groups having $\pi$ values of 0·50, whereas Equation (9), having no optimum value for $\pi$, suggests that very bulky groups will result in diminished activity. Whereas both equations predict similar activities for many substituents, in those cases where the group is substantially electronegative ($F > 2{\cdot}0$) and ($\pi/MR \geq 0{\cdot}1$), Equation (9) predicts much higher activities than Equation (10). Conversely, where $F > 0{\cdot}2$ but $\pi/MR \leq 0{\cdot}1$, Equation (10) predicts higher activities than Equation (9). Examples of compounds which would distinguish between the equations in this way are given in Table 3, and their preparation would resolve this problem. One such compound ($R = CF_3$, Table 2) has been prepared and suggests that Equation (10) represents the data better than Equation (9).

Recently we have described [10] the electronic effects of a 9α-substituent on the cortisol molecule, as determined by CNDO/2 calculations. In the present case one can only say that the 6-substituent must exert an analogous effect, most probably through the conjugated ketone system. This effect may result in enhanced cytoplasmic receptor binding, as we have described [14] in the cortisol series. The effect due to $\pi$, on the other hand, may well be caused by changes in drug transport and distribution. For example, there is precedent for this in the testosterone esters, where activity is readily correlated with partition coefficient [15].

The 6-methyl compound is quite different from all of the other compounds and would be predicted to

Table 2

Progestational Activity and Substituent Constants of Progesterone Derivatives




| R | Obsd Relative Activity[a] | Calcd Relative Activity[b] | Calcd Relative Activity[c] | Obsd log A | Calcd log A[c] | Calcd[b] log A | F | π | R | MR |
|---|---|---|---|---|---|---|---|---|---|---|
| $CH_3$[d] | 91 | 0.9 | 1.7 | 1.96 | 0.22 | -0.04 | -0.04 | 0.50 | -0.13 | 5.65 |
| Cl | 77 | 30.2 | 47.9 | 1.89 | 1.68 | 1.48 | 0.41 | 0.71 | -0.15 | 6.03 |
| F | 55 | 28.2 | 35.5 | 1.74 | 1.55 | 1.45 | 0.43 | 0.14 | -0.34 | 0.92 |
| Br | 42 | 30.9 | 51.3 | 1.62 | 1.71 | 1.49 | 0.44 | 0.86 | -0.17 | 8.88 |
| $N_3$ | 20 | 13.8 | 6.8 | 1.30 | 0.83 | 1.14 | 0.30 | 0.46 | -0.13 | 10.20 |
| OMe | 14 | 5.1 | 2.6 | 1.15 | 0.41 | 0.71 | 0.26 | -0.02 | -0.51 | 7.87 |
| SCN | 12 | 21.9 | 5.2 | 1.08 | 0.72 | 1.34 | 0.36 | 0.41 | 0.19 | 13.40 |
| $CF_3$ | 11 | 18.6 | 67.6 | 1.04 | 1.83 | 1.27 | 0.38 | 0.88 | 0.19 | 5.02 |
| CN | 6 | 4.5 | 5.0 | 0.78 | 0.70 | 0.65 | 0.51 | -0.57 | 0.19 | 6.33 |
| OEt | 1 | 7.1 | 0.4 | 0.00 | -0.36 | 0.85 | 0.22 | 0.38 | -0.44 | 12.47 |
| H | 1 | 0.7 | 1.5 | 0.00 | 0.18 | -0.17 | 0.0 | 0.00 | 0.00 | 1.03 |
| CHO | 1 | 0.6 | 1.0 | 0.00 | 0.01 | -0.23 | 0.31 | -0.65 | 0.13 | 6.88 |
| OAc | 0.2 | 1.4 | 0.8 | -0.70 | -0.10 | 0.14 | 0.41 | -0.64 | -0.07 | 12.47 |
| NHAc | 0.1 | 0.1 | 0.1 | -1.00 | -0.96 | -1.22 | 0.28 | -0.97 | -0.26 | 14.93 |

[a]Relative activity (progesterone = 1) from ref. 1. [b]Calculated using equation (10). [d]Not included in the derivation of the equations. [c]Calculated using equation (9).

Table 3

Calculated Activities of Potential 6-Substituted-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate Derivative

| 6-Substituent | Calcd Rel Act (Eq. 10) | Calcd Rel Act (Eq. 9) | Calcd log A (Eq. 10) | Calcd log A (Eq. 9) | F | π | R | MR |
|---|---|---|---|---|---|---|---|---|
| $SO_2C_6H_5$ | 94.9 | 0.6 | 1.98 | -0.21 | 0.56 | 0.27 | 0.18 | 33.2 |
| $OC_6H_5$ | 0.0 | 20.2 | -1.33 | 1.31 | 0.34 | 2.08 | -0.35 | 27.68 |
| $SF_5$ | 32.9 | 239.7 | 1.52 | 2.38 | 0.57 | 1.23 | 0.15 | 9.89 |
| $SCF_3$ | 2.5 | 48.3 | 0.39 | 1.68 | 0.35 | 1.44 | 0.12 | 13.81 |

have an activity of only about 1 by Equations (9) and (10). This enormous difference (4 standard deviations) from the observed activity (91) justifies the exclusion of this compound from the derivation of the equations and is a clear indication that the methyl group is acting by a different mechanism from all of the other substituents. The enhancing action could not involve the formation of an active ordinary metabolite of the methyl group itself, since such metabolic products ($CH_2OH$, CHO and COOH) would be predicted (or are found) to be even less active. The activation could, however, be brought about through the inhibition of enzymatic destruction of the parent steroid or through the formation of an extraordinary metabolite. It is not unexpected that different substituents may exert their effect by different means, and one of the strengths of quantitative structure activity relationships studies is that these situations are readily apparent. The C=NOMe derivative was not included in the analysis because we had no estimates of $\pi$ and $F$ for this group.

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