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References

(1) G. R. Pettit, E. I. Saldana, and E. Lehto, *Lloydia*, in press (paper 35).
(2) G. R. Pettit, M. R. Chamberland, and B. Green, *Can. J. Chem.*, **45**, 1555 (1967); G. R. Pettit and S. K. Gupta, *ibid.*, **45**, 1561 (1967).
(3) G. R. Pettit and B. J. Danley, *Can. J. Chem.*, **46**, 792 (1968).
(4) V. W. Werner, *J. Prakt. Chem.*, **37**, 154 (1968).
(5) S. G. Kuznetsov, N. M. Libman, and V. A. Voldovq, *Zh. Org. Khim.*, **6**, 442 (1970); D. A. Simon, R. L. Salvador, and C. Gaston, *J. Med. Chem.*, **13**, 1249 (1970).
(6) R. I. Geren, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, Vol. 3, No. 2 (Sept 1972).
(7) G. R. Pettit, J. A. Settepani, and R. A. Hill, *Can. J. Chem.*, **43**, 1792 (1965).

A Quantitative Reexamination of Structure–Activity Relationships in the Δ^6-6-Substituted Progesterone Series

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In a recent article Teutsch, *et al.*,[1] 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.

By contrast, in our own quantitative structure–activity relationship (QSAR) study of 9α-substituted cortisol derivatives,[2] we showed, for the first time, that the multiparameter regression technique (for a review, see ref 3) can be applied to steroids. In the present report we describe the application of this method to the foregoing progesterone derivatives (Table I) and the obtainment of a quantitative relationship differing from the conclusions drawn by Teutsch, *et al.*[1]

Instead of selecting and deriving new estimates of physical properties, we used the stochastic method utilizing known physicochemical parameters for the hydrophobic bonding power π,[4] the inductive and resonance effects $\mathcal{F}$ and $\mathcal{R}$,[5] and the size of the substituent (molar refraction, MR). As has been pointed out,[6] these parameters have been determined for a wide variety of substituents, an important consideration in using the relationship in predicting new candidates for synthesis. Although some of these parameters have been derived from aromatic systems, they are suitable for the present study in which the substituent is attached to a conjugated unsaturated carbon. From the data in Table I we derived eq 1–10 by the method of least squares. 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 15 compounds in the series, two (R = CH_3 and R = C=NOMe) could not be included in the regression analysis, for reasons to be discussed later.

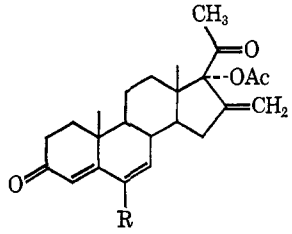
In the single parameter equations (1–3) the one with π gives the best result, accounting for over 50% of the variance in the data. It is noteworthy that eq 1 and 3, involving the steric and electronic parameters, each account for less than 20% of the variance. These were the features considered to be *most* important by Teutsch, *et al.*[1] Again, these authors considered resonance to be an important component of the electronic effect, but eq 4 indicates that this factor ($\mathcal{R}$) has no influence on activity.

Looking at the two parameter equations (5–8) it is seen that only the ones involving π (eq 5, 7, 8) have high r values. Equation 7, embodying π and the electronic term $\mathcal{F}$, gives the best two term result, accounting for 69% of the variance.

The three parameter equations (9 and 10) both give high correlations. Both equations satisfy the F test[7] at the 0.005 level, having F values of 11.6 and 11.4, respectively. In these equations, the π^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. We are inclined to favor eq 10 since π^2 must ultimately have importance in any series involving π itself. 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, eq 10 predicts that activity reaches a maximum with the group having π values of 0.50, whereas eq 9, hav-

	n	s	r^2	r	
$\log A = 1.45\ (\pm 1.15) - 0.09\ (\pm 0.12)$ MR	13	0.875	0.198	0.444	(1)
$\log A = 0.60\ (\pm 0.40) + 1.14\ (\pm 0.66)\ \pi$	13	0.645	0.564	0.751	(2)
$\log A = -0.29\ (\pm 1.55) + 2.94\ (\pm 4.39)\ \mathcal{F}$	13	0.892	0.165	0.406	(3)
$\log A = -0.41\ (\pm 1.74) + 3.15\ (\pm 4.72)\ \mathcal{F} - 0.52\ (\pm 2.54)\ \mathcal{R}$	13	0.926	0.182	0.427	(4)
$\log A = -1.11\ (\pm 0.84) - 0.06\ (\pm 0.09)$ MR $+ 1.04\ (\pm 0.65)\ \pi$	13	0.611	0.644	0.803	(5)
$\log A = 0.43\ (\pm 1.61) - 0.10\ (\pm 0.11)$ MR $+ 3.31\ (\pm 3.95)\ \mathcal{F}$	13	0.790	0.405	0.636	(6)
$\log A = -0.24\ (\pm 1.01) + 1.10\ (\pm 0.60)\ \pi + 2.54\ (\pm 2.86)\ \mathcal{F}$	13	0.574	0.686	0.828	(7)
$\log A = 0.79\ (\pm 0.63) + 1.13\ (\pm 0.68)\ \pi - 0.53\ (\pm 1.35)\ \pi^2$	13	0.652	0.595	0.771	(8)
$\log A = 0.26\ (\pm 1.01) - 0.07\ (\pm 0.07)$ MR $+ 0.97\ (\pm 0.53)\ \pi + 2.84\ (\pm 2.50)\ \mathcal{F}$	13	0.489	0.795	0.892	(9)
$\log A = -0.17\ (\pm 0.88) + 1.06\ (\pm 0.52)\ \pi + 3.46\ (\pm 2.68)\ \mathcal{F} - 1.05\ (\pm 1.11)\ \pi^2$	13	0.492	0.792	0.890	(10)

Table I. Progestational Activity and Substituent Constants of Progesterone Derivatives



R	Obsd rel act.[a]	Calcd rel act.[b]	Calcd rel act.[c]	Obsd log A	Calcd log A^c	Calcd log A^b	$\mathcal{F}$	π	$\mathcal{R}$	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 eq 10. [c]Calculated using eq 9. [d]Not included in the derivation of the equations.

Table II. Calculated Activities of Potential 6-Substituted 16-Methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate Derivatives

6 substituent	Calcd rel act. (eq 10)	Calcd rel act. (eq 9)	Calcd log A (eq 10)	Calcd log A (eq 9)	$\mathcal{F}$	π	$\mathcal{R}$	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

ing no optimum value for π, 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 ($F > 0.2$) and (π/MR ≥ 0.1), eq 9 predicts much higher activities than eq 10. Conversely, where $F > 0.2$ but π/MR ≤ 0.1, eq 10 predicts higher activities than eq 9. Examples of compounds which would distinguish between the equations in this way are given in Table II, and their preparation would resolve this problem. One such compound (R = CF_3, Table I) has been prepared and suggests that eq 10 represents the data better than eq 9.

Recently we have described[8] 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† in the cortisol series. The effect due to π, 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 the partition coefficient.[9]

The 6-methyl compound is quite different from all of the other compounds and would be predicted to have an activity of only about 1 by eq 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 QSAR is that these situations are readily apparent. The C=NOMe derivative was not included in the analysis because we had no estimates of π and $\mathcal{F}$ for this group.

†M. E. Wolff and J. Baxter, manuscript in preparation.

References

(1) G. Teutsch, L. Weber, G. Page, E. L. Shapiro, H. L. Herzog, R. Neri, and E. J. Collins, *J. Med. Chem.*, 16, 1370 (1973).
(2) M. E. Wolff and C. Hansch, *Experientia*, 29, 1111 (1973).
(3) C. Hansch in "Drug Design," E. J. Ariens, Ed., Academic Press, New York, N. Y., 1971, Chapter 2, pp 271–342.
(4) (a) C. Hansch and S. M. Anderson, *J. Org. Chem.*, 32, 2583 (1967); (b) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(5) C. G. Swain and E. C. Lupton, *J. Amer. Chem. Soc.*, 90, 4328 (1968).

(6) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nakaitani, and E. J. Lien, J. Med. Chem., 16, 1207 (1973).
(7) N. R. Draper and H. Smith, "Applied Regression Analysis," Wiley, New York, N. Y., 1966.
(8) P. A. Kollman, D. Giannini, W. L. Duax, S. Rothenberg, and M. E. Wolff, J. Amer. Chem. Soc., 95, 2869 (1973).
(9) K. C. James, Experientia, 28, 479 (1972).

Radioprotective Activity of p-Aminopropiophenone. A Structure–Activity Investigation†

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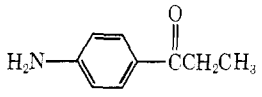
p-Aminopropiophenone (**1**) is well known for its radioprotective action which has generally been attributed to the anoxia associated with high levels of methemoglobin (metHb) following administration of **1** to animals.[1] However, certain lines of evidence suggest[2,3] that the radioprotection of **1** may not be related to metHb levels. Lack of any systematic structure–activity survey of the radioprotective effect of **1** prompted the present investigation of this relatively simple molecule. To this end a number of compounds related to **1** have been examined in an effort to sort out those features associated with radioprotection. The derivatives chosen for evaluation represent alterations in the amino function, variations in the acyl chain length, and a variety of para-substituted anilines. Within this group of substances, the only consistent structural feature required for radioprotection was the presence of a free amine or hydroxylamine group. While no evidence was obtained to support a general mechanism for the radioprotective action of **1**, it was found that in certain compounds (**12**, **13**, and **16**) metHb-forming ability was not necessary for radioprotective action.



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Results and Discussion

Biological. The structural relationships between **1** and the various derivatives investigated in this work may, for convenience, be divided into three groups: (1) changes in the nitrogen function (Table I); (2) changes in the alkyl chain length of the ketone function (Table II); (3) changes in the substituent para to the amino group (Tables III and IV). In these experiments usually more than one dose was tried, but only the dose giving maximum protection is shown in the tables.

Generally, alteration of the amino group led to less radioprotective compounds although appreciable toxicity characterized the nitroso (**3**) and *N,N*-dimethyl (**7**) derivatives (Table I). In view of the fact that free radicals have been implicated in radiation toxicity,[1] it was anticipated that the nitroso derivative **3** might show good radioprotection since the nitroso function is known to be a good radical scavenger.[4] However, this did not prove to be the case.

Several years ago *p*-aminoacetophenone (**8**) was tested to a limited extent and found to exhibit some radioprotection although less than *p*-aminopropiophenone.[5] Furthermore, it has been reported that *p*-aminobutyrophenone and *p*-aminovalerophenone (**9**) also showed radioprotection.[6,7] It was, therefore, of interest to examine the effect on radioprotective activity of extending the alkyl chain of the ketone moiety. Thus, compounds having an alkyl chain of 4, 5, and 6 carbons (as compared to 2 carbons in **1**) were prepared and evaluated. The results are shown in Table II. While all three compounds (**9**, **10**, and **11**) showed increased toxicity, only the *p*-pentanoyl- (**9**) and *p*-hexanoyl- (**10**) anilines exhibited radioprotective activity and, in fact, were more radioprotective than **1**.

Replacement of the ketone function by alkyl chains of different lengths gave a different pattern of radioprotective activity (Table III). In contrast to the ketones, the *p*-alkylanilines with shorter chains showed greater activity than the longer chain analogs. More interesting perhaps is the observation that *p*-methyl- and *p*-ethylaniline (**12** and **13**) and *o*-propylaniline (**16**) exhibited good radioprotection but produced essentially no metHb.‡ On the other hand, *p*-*n*-butylaniline (**15**), which produced high levels of metHb, was a poor radioprotective agent. These compounds, then, represent the first derivatives of **1** which effectively separate radioprotection and metHb-forming ability. Surprisingly, *p*-methoxyaniline (**20**), which is isosteric with *p*-ethylaniline (**13**), showed poor radioprotection as did *p*-ethoxyaniline (**21**). The *p*-trifluoromethyl- (**17**) and *p*-cyano- (**18**) anilines provided moderate protection against radiation. It is obvious, then, that the carbonyl function of **1** is not necessary for the molecule to be radioprotective. However, it is not possible at this time to be certain that **1** and the *p*-alkylanilines share a common mechanism for radioprotection.

Finally, it was of interest to compare the radioprotective activity of a series of *p*-aminobenzoic acid esters, which are isosteric with the *p*-aminophenones. The results are summarized in Table IV. The parent acid **22** gave poor protection, whereas the methyl and ethyl esters **23** and **24** were good radioprotectors. Activity dropped off with the propyl ester **25**, and the butyl and isobutyl esters **26** and **27** were poor protectors. These results appear to approximate more closely the trend seen with the *p*-alkylanilines (Table III) and are in contrast to the pattern observed with the ketones (Table II) in which radioprotection is associated with the corresponding longer chain lengths.

During the course of this work, it came to our attention that tetrahydrofolic acid (THFA) had been shown by three different groups of workers[8-10] to be an effective radioprotector even when administered to the animals after irradiation. The title compound (**1**), structurally similar to the *p*-aminobenzoyl moiety of THFA, is known to be effective only if given before radiation treatment. It seemed reasonable, then, to suspect that the radioprotective mechanism of **1** might be associated in some way with THFA metabolism. However, if THFA and the radioprotection of **1** were related, it would be reasonable to expect a mono-N-substituted derivative of **1**, being structurally closer to THFA, would retain good radioprotective action; in fact, the *N*-methyl derivative of **1** (**6**) was a poor protector. Furthermore, *p*-aminobenzoyl-L-glutamic acid (**28**), which even more closely resembles THFA, showed no radioprotective action in our experiments. It seems unlikely, then, that there is any obvious relationship between the radioprotective effect of **1** and THFA metabolism.

These results show no consistent structural requirements for good radioprotective activity of aniline derivatives save for a free amine or hydroxylamine function. Compared to **1**, the radioprotective activity of these compounds may be increased or decreased depending on the ortho or para substitutent, but with no apparent common

†A preliminary account of this work was presented at the 21st Annual Meeting of the Radiation Research Society in St. Louis, Mo., on May 1, 1973.

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‡A complete investigation of the metHb-producing ability and toxicity of these compounds is in progress and results will be published later.