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GLUCOCORTICOID POTENCY AND PARACHOR

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SUMMARY

The potencies of some glucocorticoids by several assay methods show some interesting correlations with their parachors. Parachor is an additive and constitutive property of a molecule and is related to the molar volume and the surface tension. The parachor values of the steroids were calculated from their constituent atoms and bonds. Parachor and the Hansch hydrophobic constant π were compared for their relative effectiveness in correlations for the anti-inflammatory potency of 9α-substituted cortisol analogs, using multiple-regression analysis. The relative binding affinities of various steroids to specific cytosol receptor proteins showed a correlation with the steroidal parachors. The ability of glucocorticoids to cause the induction of three enzymes: glutamine synthetase, tyrosine aminotransferase and alkaline phosphatase and to stimulate the growth of a line of mouse lymphoma cells in culture, show similar correlations with steroid parachors.

INTRODUCTION

Correlations between the biological activities and chemical structures of steroids are important in drug design. The multiparameter regression technique of Hansch and co-workers [1, 2] was applied for the first time to an analogous series of monosubstituted steroids, 9α-substituted cortisol derivatives by Wolff and Hansch [3]. They reported that the correlation of the anti-inflammatory potency of 9α-substituted cortisol analogs in the rat liver glycogen deposition assay with the Hansch hydrophobic constant π was low; but improved significantly by the addition of other physicochemical parameters, such as inductive effect (σ_I), molar refractivity (P_E) and steric effect (E_S). We have examined the usefulness of the parachor on such correlations. The parachor is related to the molar volume and the surface tension [4], and like molar refractivity is a measure of effective molecular size. Previously we had shown the usefulness of the parachor as a parameter for structure–activity correlations for several drug classes [5]. We have found some interesting correlations between the parachor-values of steroids and some biological activities, such as lysosome stabilization and anti-inflammatory potency [6, 7]. In this paper, we present correlations for 9α-substituted cortisol derivatives. Other correlations with parachor that we have examined include Ringler's data [8] on the comparison of glucocorticoid potencies for an extended series of compounds and the potencies of some synthetic experimental steroids as reported by Applezweig [9]. The data of eight different studies [10–17] on the relative steroid binding affinities of some purified receptor proteins from a number of different biological sources were examined for a correlation with the parachors of the steroids. The ability of glucocorticoids to induce glutamine synthetase [18], tyrosine aminotransferase [19] and alkaline phosphatase [20] in different biological systems show similar correlations with the steroid parachors. There is also a correlation between the parachors of the steroids and their relative stimulatory effect on the growth of a line of mouse lymphoma cells [21]. Unlike the Wolff and Hansch studies [3, 22] on steroids with substitutions at only one position, our analyses include steroids of various structural modifications at several positions of the steroid nucleus.

METHODS

Trivial names, systematic names, symbols used in the figures and parachor values of the steroids reported in this paper are given in Table 1. Letter

Table 1. Trivial names, systematic names, symbols used in Figures and parachor values of the steroids used in this paper

Trivial name	Systematic name	Symbol used in Figures	Parachor value
Estradiol-17β	1,3,5(10)-Estratriene-3,17β-diol	A	623.7
Androstenedione	4-Androstene-3,17-dione	B	671.1
Testosterone	17β-Hydroxy-4-androsten-3-one	C	680.8

Table 1 (*Continued*)

Trivial name	Systematic name	Symbol used in Figures	Parachor value
Progesterone	4-Pregnene-3,20-dione	D	751.3
Deoxycortico-sterone	21-Hydroxy-4-pregnene-3,20-dione	E	765.6
Corticosterone	11β,21-Dihydroxy-4-pregnene-3,20-dione	F	781.2
Cortexolone (11-deoxycortisol)	17,21-Dihydroxy-4-pregnene-3,20-dione	G	782.5
Cortisone	17,21-Dihydroxy-4-pregnene-3,11,20-trione	H	787.3
Cortisol	11β,17,21-Trihydroxy-4-pregnene-3,20-dione	I	796.8
Aldosterone	18,11-Hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al	J	802.5
Tetrahydro-cortisol	3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	K	823.8
Dexamethasone	9α-Fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	L	834.1
Estrone	1,3,5(10)-Estratriene-3-hydroxy-17-one	1	614.2
Diethylstilbestrol	α,α'-Diethyl-stilbenediol	2	628.7
Tetrahydroxy-pregnenolone	3β,11β,17,21-Tetrahydroxy-5-pregnen-20-one	3	810.5
19-Nortestosterone	19-Nor-17β-hydroxy-4-androsten-3-one	4	640.8
4-Androsten-3-one	4-Androsten-3-one	5	665.1
Dehydrotestos-terone	17β-Hydroxy-1,4-androstadien-3-one	6	667.2
Androstan-17-one	Androstan-17-one	7	679.1
Dehydroepi-androsterone	3β-Hydroxy-5-androsten-17-one	8	680.8
Androstanedione	Androstane-3,17-dione	9	685.2
Androsterone	3α-Hydroxy-5α-androstan-17-one	10	694.1
Etiocholanolone	3β-Hydroxy-5α-androstan-17-one	11	694.1
Dihydrotestosterone	17β-Hydroxy-5β-androstan-3-one	12	694.1
Methyltestosterone	17α-Hydroxy-17-methylandrosten-3-one	13	720.8
Pregnenolone	3β-Hydroxy-5-pregnen-20-one	14	747.1
Ketoprogesterone	4-Pregnen-3,11,20-trione	15	757.8
Pregnanolone	3α-Hydroxy-5β-pregnan-20-one	16	760.8
Hydroxypregnenolone	3α,17α-Dihydroxy-5-pregnen-20-one	17	761.4
17α-Hydroxy-progesterone	17α-Hydroxy-4-pregnene-3,20-dione	18	765.6
17β-Hydroxy-progesterone	17β-Hydroxy-4-pregnene-3,20-dione	19	765.6
Deoxycorticosterone acetate	21-Hydroxy-4-pregnene-3,20-dione 21-acetate	20	851.6
Prednisone	17,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione	21	774.0
21-Deoxycortisol	11β,17-Dihydroxy-4-pregnene-3,20-dione	22	782.5
Prednisolone	11β,17,21-Trihydroxy-1,4-pregnadiene -3,20-dione	23	783.5
Tetrahydrodeoxy-corticosterone	3α,21-Dihydroxy-5β-pregnan-20-one	24	792.6
Fluprednisolone	6α-Fluoro-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	25	794.1
9α-Fluoro-prednisolone	9α-Fluoro-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione	26	794.1
11-epicortisol	11α,17,21-Trihydroxy-4-pregnene-3,20-dione	27	796.8
Reichstein's substance E	4-Pregnene-11β,17,20β,21-tetrol-3-one	28	796.8
Reichstein's substance U	4-Pregnene-17,20β,21-triol-3,11-dione	29	805.0
9α-Fluorocortisol	9α-Fluoro-4-pregnene-11β,17,21-triol-3,20-dione	30	807.4
Tetrahydro-corticosterone	3α,11β,21-Trihydroxy-5β-pregnan-20-one	31	808.2
Tetrahydro-11-deoxycortisol	3α,17,21-Trihydroxy-5β-pregnan-20-one	32	809.5
Triamcinolone	9α-Fluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	33	809.6
5α-Dihydrocortisol	11β,17,21-Trihydroxy-5α-pregnane-3,20-dione	34	810.1

Table 1 (*Continued*)

Trivial name	Systematic name	Symbol used in Figures	Parachor value
5β-Dihydrocortisol	11β,17,21-Trihydroxy-5β-pregnane-3,20-dione	35	810.1
20α-Hydroxycortisol	11β,17,20α,21-Tetrahydroxy-4-pregnen-3-one	36	810.5
20β-Hydroxycortisol	11β,17,20β,21-Tetrahydroxy-4-pregnen-3-one	37	810.5
2α-Hydroxycortisol	2α,11β,17,21-Tetrahydroxy-4-pregnen-3,20-dione	38	811.1
Tetrahydrocortisone	3α,17α,21-Trihydroxy-5β-pregnane-11,20-dione	39	814.3
Flucinolone	6α,9α-Difluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	40	820.3
6α-Methylprednisolone	11β,17,21-Trihydroxy-6α-methyl-1,4-pregnadiene-3,20-dione	41	823.5
Triamcinolone acetonide	9α-Fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione acetonide	42	926.7
Flucinolone acetonide	6α,9α-Difluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione acetonide	43	937.2
Spironolactone	17-Hydroxy-7-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone, 7-acetate	44	912.3
Corticosterone acetate	11β,21-Dihydroxy-4-pregnene-3,20-dione 21-acetate	45	867.2
Cortisol hemisuccinate	11β,17,21-Trihydroxy-4-pregnene-3,20-dione 21-hemisuccinate	46	982.3
Tetrahydrocortisol acetate	3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one 21-acetate	47	909.8

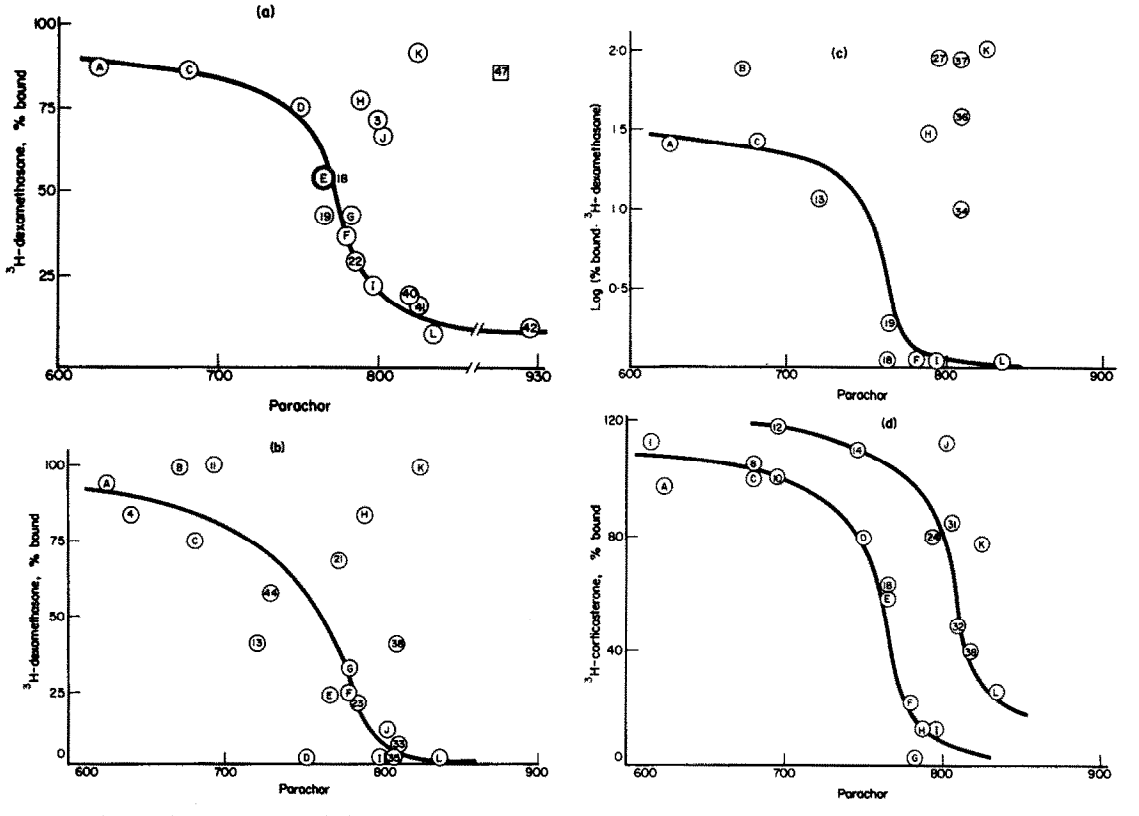


Fig. 1. The competitive binding of various steroids to specific cytosol receptor preparations from four different sources, plotted as a function of steroid parachor. The four graphs represent competition for: (1a) dexamethasone binding to rabbit fetal lung receptor, (1b) dexamethasone binding to human lymphoblast receptor, (1c) dexamethasone binding to rat hepatoma receptor, and (1d) corticosterone binding to chicken liver receptor. The biological data are from Giannopoulos [10] for Fig. 1a, Lippman *et al.* [11] for Fig. 1b, Baxter and Tomkins [12] for Fig. 1c and Crochet and Chambaz [12] for Fig. 1d. The arbitrary symbols used to designate steroids are explained in Table 1, steroids common to most of the studies are denoted by letter symbols, others by number symbols. The ordinate for each plot represents relative binding for the steroids expressed as a percentage or $\log_{10}$ (percentage) of the radioactive steroid remaining bound in the presence of the various unlabelled steroids.

symbols were used for those steroids which had been reported in most of the studies whose data were used in Figs. 1–3. Number symbols were used for steroids reported in one or two of these studies. Other biological data were obtained from Wolff and Hansch [3] for Table 2, from Ringler *et al.* [8] for Table 3, from Applezweig [9] for Table 4, Moscona and Piddington [18] for Fig. 3a, Samuels and Tomkins [19] for Fig. 3b, Melnykovych and Bishop [20] for Fig. 3c and Harris [21] for Fig. 3d.

From the data in the Tables 2–4, the regression equations were derived *via* the method of least squares, using a program of the Institute of Computer Science, University of Guelph (SPSS, version 5). In these equations, n is the number of data points used in the regression, r is the correlation coefficient, s is the standard deviation and F is Snedecor's variance ratio. The equations 1a–1q from the data in the Table 2 were derived in an attempt to show the relative effectiveness of the parachor (P) and the Hansch hydrophobic constant π. All other equations from the data in the Tables 3–4 show the correlation of parachor and glucocorticoid potencies by different assay methods. The values of the Hansch hydrophobic constant π, inductive effect (σ_I), molar refractivity (P_E) and steric effect (E_S) were taken from ref. 3 and the references mentioned therein. "Computation of parachors:" The parachor values of organic compounds can be obtained in two ways: (i) from standard tables by summation of the parachors of all the atoms and other structural features occurring in the compound; and (ii) by experimental determinations of surface tension and density. We have used Quayle's table of recommended parachors [23] for the calculation of all parachors reported in this paper. With the help of the parachor tables, it is possible to calculate the molecular parachor value of any compound if the chemical structure of the compound is known.

RESULTS AND DISCUSSION

Table 2 shows a comparison of the use of parachor, Hansch hydrophobic constant π, σ_I, P_E and E_S using the data of Wolff and Hansch [3] in structure–activity correlations for seven 9α-substituted cortisol derivatives. The various regression equations show that in single parameter equations (1a–1e) parachor gives a better fit than the Hansch π; and the other three terms: σ_I, P_E and E_S give fairly similar fit to that of parachor. Of the two-parameter equations (1f–1m), the combination of the Hansch π and σ_I (1f) is poorer than the combination of parachor and σ_I (1g), which

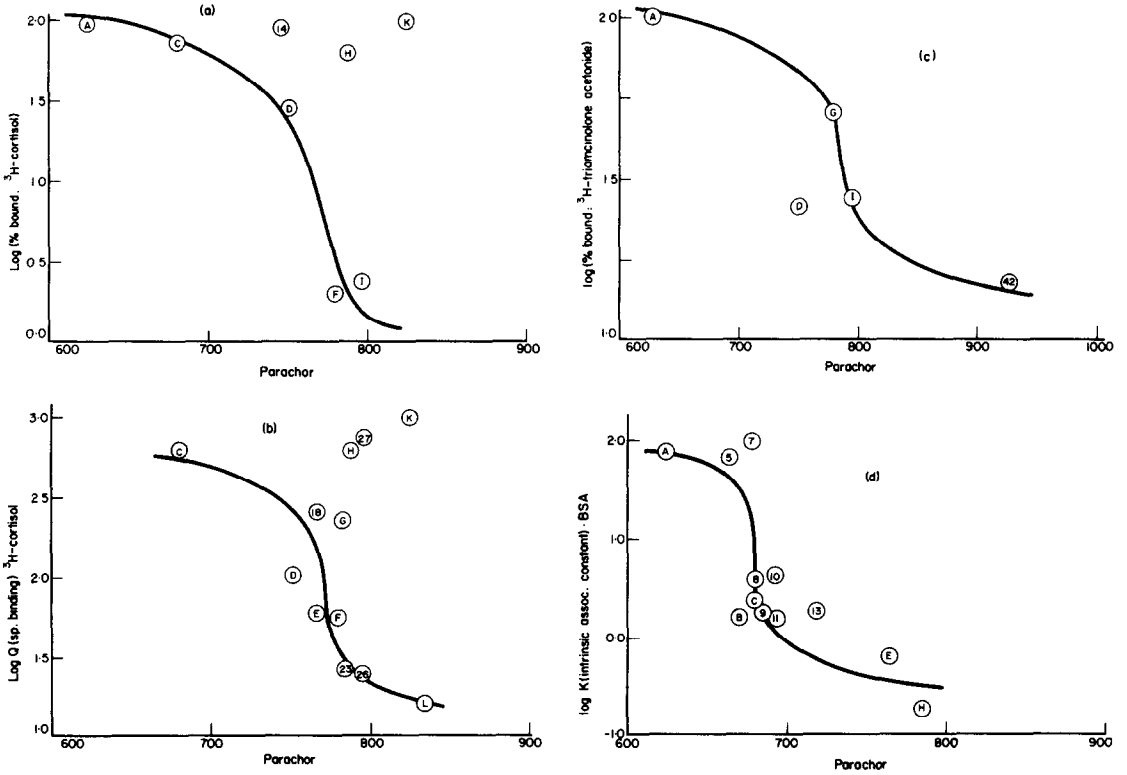


Fig. 2. The competitive binding of various steroids to specific cytosol receptor proteins (2a–2c) and to bovine serum albumin (2d), plotted as a function of steroid parachor. The four graphs represent competition for: (2a) cortisol binding to rat liver receptor, (2b) cortisol binding to rat thymus receptor, (2c) triamcinolone acetonide binding to rat mammary gland receptor, and (2d) non-specific binding of steroids to BSA. Biological data are from Beato *et al.* [14] for Fig. 2a, Munck and Wira [15] for Fig. 2b, Gardner and Wittliff [16] for Fig. 2c and Eik-Nes *et al.* [17] for Fig. 2d. The letter and number symbols for steroids are explained in Table 1. The data are plotted as in Fig. 1.

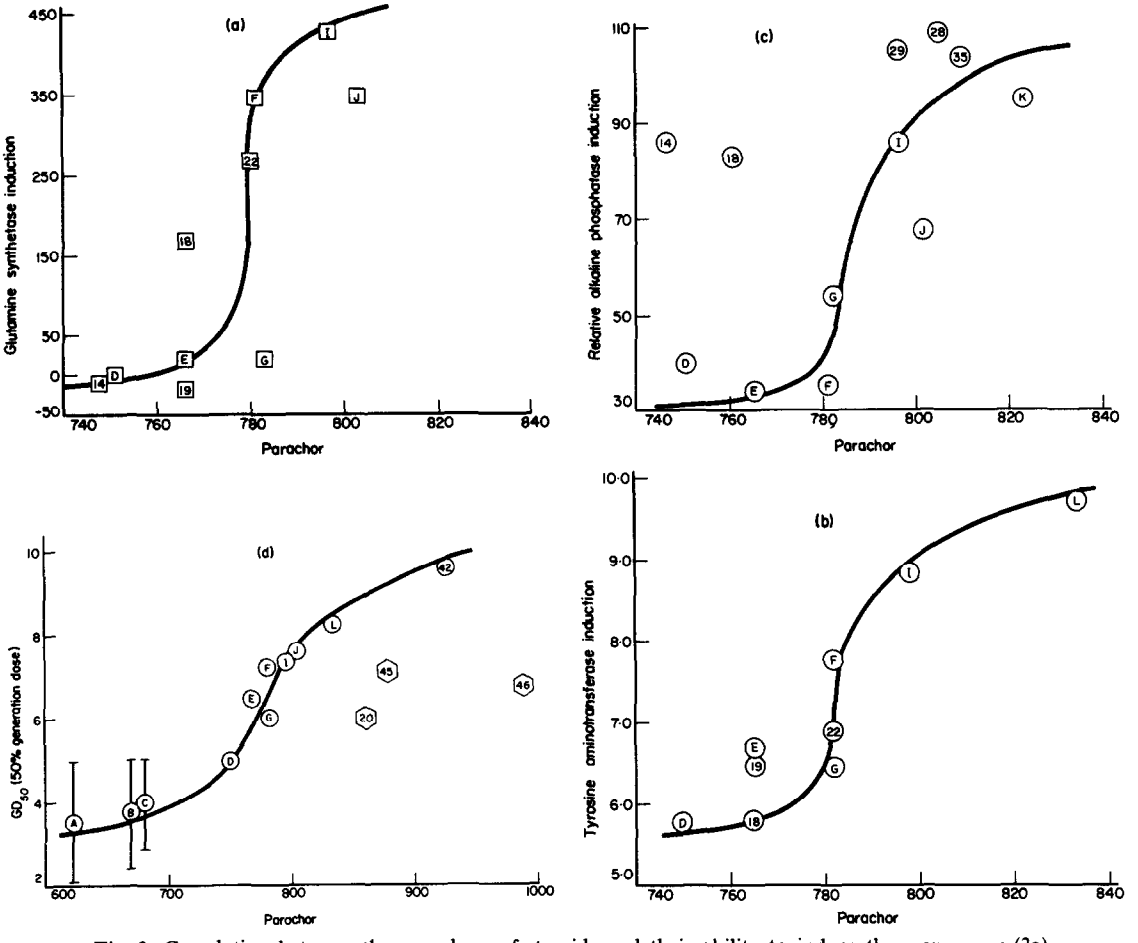
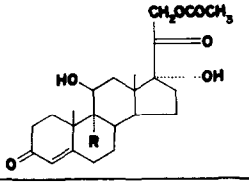


Fig. 3. Correlation between the parachors of steroids and their ability to induce three enzymes: (3a) glutamine synthetase, (3b) tyrosine aminotransferase and (3c) alkaline phosphatase, compared with the stimulatory effect of steroids on the growth of a line of mouse lymphoma cells in culture (3d). The biological data for Figs. 3(a–d) are from ref. 18, 19, 20, 21 respectively. The symbols for the steroids in the figures are explained in Table 1. GD_{50} (50% generation dose) is an index of relative effect on cell growth (Fig. 3d).

Table 2. Liver glycogen deposition activity and substituent constants for 9α-substituted cortisol derivatives.



9α-Substituent group	Observed[a] relative activity: (A)	log of observed activity: (log A)	Hansch π[b]	Parachor (P)[c]	σ_I[d]	log.P_E[e]	log E_S[f]
F	10.7	1.03	-0.17	26.1	0.52	1.20	0.78
Cl	4.7	0.67	0.39	55.2	0.47	5.96	0.27
Br	0.3	-0.52	0.60	68.0	0.45	8.86	0.08
I	0.1	-1.00	1.00	90.3	0.38	13.90	-0.16
OH	0.2	-0.70	-1.16	29.8	0.25	2.62	0.69
H	1.0	0.00	0.00	15.5	0.00	1.10	1.24
CH_3	0.1	-1.00	0.50	55.5	0.00	5.72	0.00

[a]Relative activity (cortisol acetate = 1) from ref.3,24. [b]From ref.3. [c]From ref.23. [d]From ref.25. [e]From ref.26. [f]From ref.27.

Table 2 (continued):

Equations :(here, the number of cases, n = 7):	r	s	F
(1a) log A = -0.1799 - 0.2248 π	0.19	0.87	0.20
(1b) log A = 0.5335 - 0.0154 P	0.50	0.77	1.68
(1c) log A = -0.7408 + 1.7707 σ_I	0.48	0.78	1.49
(1d) log A = 0.3219 - 0.0959 log P_E	0.55	0.74	2.13
(1e) log A = -0.5564 + 0.8190 log E_S	0.51	0.76	1.75
(1f) log A = -0.7256 - 0.2965 π + 1.8857 σ_I	0.54	0.83	0.83
(1g) log A = 0.0975 - 0.0226 P + 2.6484 σ_I	0.84	0.53	4.93
(1h) log A = 0.5992 + 0.5821 π - 0.1624 log P_E	0.64	0.76	1.38
(1i) log A = -0.1910 + 0.0287 P - 0.2562 log P_E	0.58	0.81	0.99
(1j) log A = -0.7268 + 0.3090 π - 1.1067 log E_S	0.55	0.83	0.85
(1k) log A = -0.1469 - 0.0059 P + 0.5272 log E_S	0.51	0.85	0.72
(1l) log A = -0.2386 + 2.5396 σ_I - 0.1298 log P_E	0.86	0.51	5.52
(1m) log A = 0.9775 + 0.5369 π - 0.0264 P	0.58	0.81	1.03
(1n) log A = 0.0726+0.764 π +2.779 σ_I -0.22 log P_E	0.96	0.33	11.30
(1o) log A = -0.265 +0.0017 P+2.529 σ_I -0.139 log P_E	0.86	0.59	2.76
(1p) log A = 0.2722 +0.5383π +0.0178 P -0.257 log P_E	0.65	0.87	0.72
(1q) log A = 0.7219 +0.8299π -0.0405 P + 3.0248 σ_I	0.96	0.34	10.58

In these equations, n is the number of cases or data points used in the regression, r is the correlation coefficient, s is the standard deviation and F is Snedecor's variance ratio.

Table 3. Biological potencies of glucocorticoid derivatives in rat and man and their correlation with their parachor values.

Steroids	Parachor: (P)	Rat liver glycogen deposition: (A_g)	Eosinopenic activity in man: (A_e)
Cortisol	796.8	1.00	1.00
Corticosterone	781.2	0.80	0.06
Prednisolone	783.5	3.90	4.00
6α-Methyl-11β-hydroxyprogesterone	805.6	2.50	0.05
6α-Methyl-9α-fluoro-21-deoxycortisol	831.8	25.00	2.00
6α-Methyl-prednisolone	823.5	11.00	5.00
6α-Methyl-9α-fluoro-prednisolone	834.1	115.00	10.00
6α-Methyl-9α-fluoro-21-deoxy-prednisolone	818.5	26.00	2.00
6α-Methyl-16α-hydroxyprednisolone	837.8	4.00	1.00
6α-Fluoro-cortisol	807.4	11.00	4.00
6α-Fluoro-prednisolone	794.1	81.00	9.00
6α,9α-Difluoro-16α-hydroxyprednisolone	819.0	112.00	5.00
9α-Fluoro-16α-methyl-prednisolone	834.1	150.00	12.00
6α,9α-Difluoro-16α-methyl-prednisolone	844.7	677.00	30.00
9α-Fluoro-cortisol	807.4	9.00	8.00
9α-Fluoro-prednisolone	794.1	55.00	20.00
9α-Fluoro-21-deoxy-prednisolone	778.5	16.00	0.50
9α-Fluoro-16α-hydroxy-prednisolone	808.4	47.00	5.00
9α-Fluoro-16α-methylprednisolone	834.1	265.00	28.00
9α-Fluoro-16α-methyl-21-deoxy-prednisolone	818.5	19.00	5.00

Table 3 (continued):

Equations : (here, the number of cases, n = 20)	r	s	F
(2a) A_g = 7814.960 log P - 22674.348	0.84	85.8	44.2
(2b) log A_g = 0.022 P - 16.822	0.57	0.7	8.6
(2c) log A_g = 29.101 log P - 83.404	0.62	0.6	11.0
(2d) A_e = 322.028 log P - 930.114	0.61	7.1	10.9
(2e) log A_e = 0.017 P - 13.671	0.46	0.7	4.8
(2f) log A_e = 21.265 log P - 61.423	0.47	0.7	5.0

In the above equations, P is the parachor of the steroid; A_g is the relative potencies of the glucocorticoids by the rat liver glycogen deposition assay and A_e is the eosinopenic potency of the glucocorticoids; the biological assay data is taken from Ringler et al.[8]. The statistical symbols, n, r, s and F, have the same meaning as in Table 2.

with its significant improvement of correlation appears to be comparable to the combination of P_E and σ_I (1–1). From the three-parameter equations (1n–1q), it appears that the effectiveness of π, P_E and σ_I combined (1n) is comparable to that of π, parachor and σ_I together (1q). The equations (1a, 1b, 1f, 1g) indicate that the parachor is a better term in these correlations than the Hansch hydrophobic constant π. The best results have been obtained with parachor and P_E separately; but the combination of these two terms (1i) does not show much improvement in the correlation. Although the best values of the correla-

Table 4. Correlation between the parachors of some synthetic steroids with unusual chemical structures and their relative glucocorticoid activity by the anti-granuloma pouch assay.

Steroids	Parachor: (P)	Anti-granuloma activity: (A_a)
Pregnane series :		
9α-Fluoro-16α-methyl-1,4,6-pregnatriene-11,17-diol-3,20-dione	820.7	20
9α-Fluoro-6,16α-dimethyl-4,6-pregnadiene-11β,17,21-triol-20-one-(3,2-c)-2'-phenylpyrazole	1050.6	2000
6,16α-Dimethyl-4,6-pregnadiene-11β,17-diol-20-one-(3,2-c)-2'-phenylpyrazole	1065.7	348
6,16α-Dimethyl-4,6-pregnadiene-11β,17-diol-20-one-(3,2-c)-2'-p-fluorophenylpyrazole	1076.3	464
6,16α-Dimethyl-4,6-pregnadiene-11β,17,21-triol-20-one-(3,2-c)-2'-phenylpyrazole 21-acetate	1173.3	551
6,16α-Dimethyl-4,6-pregnadiene-11β,17,21-triol-20-one-(3,2-c)-2'-p-fluorophenylpyrazole	1090.6	600
9α-Fluoro-16α-methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-2'-p-fluorophenyl-(3,2-c)-pyrazole	1074.5	500
16α-Methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-1'-methyl-(3,2-c)-pyrazole	923.4	1.5
16α-Methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-2'-methyl-(3,2-c)-pyrazole	923.4	5.9
16α-Methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-1'-phenyl-(3,2-c)-pyrazole	1053.3	2.0
16α-Methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-2'-phenyl-(3,2-c)-pyrazole	1053.3	60.0
16α-Methyl-11β,17,21-trihydroxy-20-oxo-4-pregneno-2'-p-fluorophenyl-(3,2-c)-pyrazole	1063.3	100
6,16α-Dimethyl-4,6-pregnadiene-11β,17-dimethyl-3,20-dione	863.4	18
9α-Fluoro-16α-methyl-4,6-pregnadiene-11β,17-diol-3,20-dione	834.0	2.0
Cortisol series :		
9α-Fluoro-6,16α-dimethyl-6-ene-cortisol	888.3	50
9α-Fluoro-6,16α-dimethyl-6-ene-cortisol 21-acetate	983.8	121
6,16α-Dimethyl-6-ene-cortisol 21-acetate	973.2	40
9α-Fluoro-16α-methyl-6-ene-cortisol 21-acetate	943.8	29
Prednisolone series :		
6,16α-Dimethyl-6-ene-prednisolone 21-acetate	959.9	71

Table 4 (continued):

Equations : (the number of cases, n = 19)	r	s	F
(3a) $\log A_a = 0.006\ P - 4.422$	0.67	0.71	13.48
(3b) $\log A_a = 0.00001\ P^2 - 0.0055\ P + 1.267$	0.67	0.73	6.44
(3c) $\log A_a = 13.916 \log P - 39.906$	0.66	0.70	13.18

In these equations, A_a is the relative biological activity of the steroids by the anti-granuloma pouch assay and P is the parachor value of the compounds. The bioassay data is from Applezweig [9]. The statistical symbols : n, r, s and F have the same meaning as in Table 2.

tion coefficients are obtained from the three-parameter equations, the evaluation of these from only seven data points used by Wolff and Hansch [3] leads to statistically uncertain estimates of the importance of the different parameters used. A major advantage of the use of parachor (P) in such correlation studies is that it is not restricted to analogous series of mono-substituted derivatives.

In Table 3, Ringler's data on the comparative glucocorticoid potencies for twenty steroids in the rat liver glycogen deposition assay and eosinopenic potency in man [8] were examined for correlation with the parachors of the steroids. The correlation obtained is fairly good considering the heterogeneous nature of this extensive list of glucocorticoids. The best values of the correlation coefficients, 0·84 and 0·61 were obtained for the linear equations containing log P, i.e., 3a and 3d respectively. A comparison between the Hansch hydrophobic constant π and the parachor is not possible because the former values for all the listed compounds are not available. In Table 4, Applezweig's data on the relative anti-granuloma potencies of some synthetic experimental steroids of unusual chemical structures [9] were subjected to a regression analysis for correlation of activity against the parachors of the compounds. Again, the correlation coefficient of 0·67 (4a) may be considered significant in view of the wide variety of structural modifications. This series clearly demonstrates the usefulness of the parachor as compared to experimentally-determined parameters, like the Hansch π, which are not readily available for these unusual synthetic steroids.

We have used similar correlations between biological activity and steroid parachor in an attempt to distinguish different types of steroid interactions. In particular we believe that parachor correlations may be a useful tool in separating "non-specific interactions", in which molar volume and hydrophobicity are important, from "specific interactions" where the limiting features of the steroid are its substituent groups. Thus in Figs. 1 (a–d) and 2 (a–c) we have plotted the data on the competitive binding of various steroids to receptor proteins of the cytosol of target tissues, as a function of the parachor values of the steroids. Fig. 2(d) shows comparable steroid binding to bovine serum albumin, long recognised to be non-specific. The steroid-protein binding data are from references [10–17]. For the purposes of comparison we have constructed arbitrary sigmoidal curves for all eight cases, to indicate patterns that might be shown by non-specific binding as in the case of BSA. We wish to emphasize the arbitrary nature of these curves, while at the same time using them as a tool to discern general correlations for a wide range of heterogeneous steroids. In view of the variety of independent studies shown here, similarities between the different plots are interesting. For any individual steroid coincidence of its data point with the curves may be quite fortuitous, or it may indicate that the measured biological activity is determined principally by non-specific binding as for bovine serum albumin binding.

The arbitrary symbols used for the steroids are given in Table 1. From Figs. 1 and 2, it appears that the common steroids: estradiol-17 (A), 4-androstene-3,17-dione (B), testosterone (C), progesterone (D), corticosterone (E), deoxycorticosterone (F), cortexolone (G), cortisol (I) and dexamethasone (L) consistently show a similar sigmoidal pattern. Cortisone (H), aldosterone (J) and tetrahydrocortisol (K) lie off the sigmoidal curve to a characteristic extent in all cases.

In these *in vitro* binding studies cortisone is not metabolized, and the lack of correlation indicates some degree of specificity at the binding site for the 11β-hydroxyl group. Another steroid which show consistent deviation from the non-specific binding pattern is 11-epicortisol (27), which again emphasizes the specific requirement for a 11β-hydroxyl group. An interesting example is aldosterone (J) which binds well to some receptors [Fig. 1b] but with low biological activity as a glucocorticoid. Among the other steroids, some of them e.g. hydroxyprogesterones (18), (19), methyltestosterone (13), androsterone (10), triamcinolone acetonide (42), usually lie near the sigmoidal line. Others have been used in only one or two of these reports; and no conclusion can be drawn about them. Some analogous steroid derivatives such as the tetrahydro compounds: tetrahydrocortisol (K), tetrahydrodeoxycorticosterone (24), tetrahydrocorticosterone (31), tetrahydrocortexolone (32), tetrahydrocortisone (38) and dihydrotestosterone (12) appear to form a sigmoidal pattern parallel to that of the parent compounds as in Fig. 1d. The tetrahydro derivatives are

biologically less potent and this is shown by the direction of the parallel shift. The overall shape of these curves indicate the tendency for the high-parachor steroids to displace the receptor-bound radioactive steroids from the binding sites more efficiently than the low-parachor steroids. We have shown that the sex hormonal steroids have low parachor values (below 770) and the corticosteroids have high parachor values (above 770). The parachor range 770–780 appears to be a critical boundary region between the two types of steroids.

Any correlation between glucocorticord receptor-binding and parachor should also be discernible as a correlation between biological activity and parachor, if the receptor-binding is rate-limiting. It is well-known that there is a good correlation between binding of steroids to receptor proteins and their cellular effects such as enzyme induction. We examined the enzyme induction potency of glucocorticoids for three different enzymes, namely, glutamine synthetase [18], alkaline phosphatase [20] and tyrosine transaminase [19], for a correlation with the parachor of the steroids, as shown in Figs. 3a, 3b and 3c respectively. Glucocorticoid effects on the growth of cultured mouse lymphoma cells [21] were also examined (Fig. 3d). Though several steroids could have been excluded from those correlations on the grounds that they are anti-inducers rather than inducers [e.g. progesterone (D) in Fig. 3b], all data points have been retained to contrast the inactive low parachor sex hormones with the potent high parachor glucocorticoids. The resultant curves are inversely related to the sigmoidal binding curves of Figs. 1 and 2. Again we see the limitation of parachor correlations in that isomers with the same parachor do not have identical biological activity, but nevertheless families of isomers tend to be clustered close to the parachor correlation trend curves.

In this paper we have attempted to describe the usefulness of the parachor as a tool for the diagnosis of non-specific interactions based mainly on hydrophobicity and molar volume, in contrast to interactions specific to polar groups or stereochemical configurations. We believe that parachor is a promising tool by which we can discern specificity in steroid effects, but that it must be used with care. It is useful because it can be calculated for a particular steroid knowing only the molecular structure of the compound, though further work is necessary to ascertain if the parachor is an accurate measure of molar volume for large complex molecules. As for all other structure–activity parameters used hitherto, parachor correlations work best on relatively homogeneous series of compounds, such as the 9α-cortisol substituents reported here. However as this paper shows, it is very useful for showing and predicting trends in biological activity for very heterogeneous groups of steroids, if caution is exercised in extrapolation and interpretation.

Correlations with parachor appear to be a useful starting point in arguments concerning specificity. It would appear from the correlations described here that steroid-binding to "specific cytosol receptors" is less specific than is frequently assumed, and that parachor is a useful way of comparing receptors with non-specific proteins such as bovine serum albumin. Other workers have raised doubts about the relationship between the specificity of steroid action and the binding to cytosol receptors. King [28] suggests that specificity resides in nuclear interactions and Hechter [29] has expressed doubt that the cytosol receptors possess sufficient capacity for discrimination between biologically active and inactive steroids. We wish to recommend the intelligent use of parachor as a tool in answering some of these questions.

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