could be the hydrophobic binding to serum constituents. Hansch[12] found similar slopes in correlating the log P values of penicillin derivatives with their in vivo activity against *Staph. aureus* in mice (-0.46 ± 0.10) and when relating log P values and hypnotic activity in mice of alkylarylureas (0.55 ± 0.09) and 5,5-alkylbarbiturates (0.57 ± 0.21).

Lien et al.,[20] when studying the acute lethal toxicity in mice of N-substituted lactams and *O*-phenyleneureas, also found a rather low slope (0.312).

Conclusion

The present data seem to confirm the usefulness of the R_m values as an expression of the lipophilic character of molecules. In particluar the extrapolation procedure could provide further interesting contributions. In this way the R_m values of several series of chemotherapeutic agents could be compared in the same chromatographic system. The highly significant correlation between R_m and log P values further points out the possibility of relationships between partition data in different systems. The slope of 0.993 in eq 1 indicates the existence of very similar lipophilic characteristics in both systems. Obviously the correctness of the extrapolated R_m values seems to depend on the parallelism of the straight lines extrapolating from the linear range of experimental data. In particular it could be important to point out that the extrapolation technique so far provided R_m values for several series of chemotherapeutic agents in the same standard system. Moreover, when the π or log P values were available equally good correlations were found with the extrapolated R_m values. Finally the results of the structure–activity relationships seem to support the possibility of a classification of drugs according to their mechanism of action at the physicochemical level. The assay of the same series of drugs in different biological systems, in vitro and in vivo, should be carried out intensively in order to get new achievements in this field.

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R_m Values of Steroids as an Expression of Their Lipophilic Character in Structure–Activity Studies

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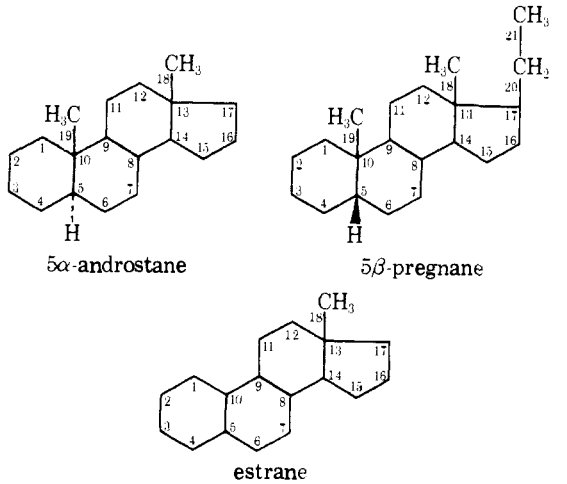
The chromatographic R_m values of three series of steroids were determined by means of a reversed-phase system. The R_m values at 45% acetone in the mobile phase were shown to be correlated with the partition coefficients in an ether–water system. However, an almost equally good correlation was found when using extrapolated R_m values. The extrapolation technique could provide a standard system. The relationship between biological data and R_m values pointed out the important role of the lipophilic character in regulating the activity of steroids. In particular, the dependence of protein binding absorption and biotransformation on lipophilic character might strongly influence the availability of steroids at the site of action.

The R_m values of testosterone derivatives and corticosteroids had been shown to be related to the Hansch π values and therefore useful in structure–activity studies.[1-4] In series of phenols,[5] penicillins and cephalosporins,[6,7] and steroids[3] the linear relationship between R_m values and acetone concentration in the mobile phase has provided the R_m values extrapolated to 0% acetone in the mobile phase. This was considered to be a standard system, where all the compounds could be compared. The purpose of the present work was to study a larger number of steroids in order to compare their lipophilic character. The possibility of the extrapolation in order to calculate the R_m values at 0% acetone in the mobile phase was taken into consideration. The partition coefficients obtained by Flynn[8] in an ether–water system were compared with the present R_m values. Finally, some data on the interaction of steroids with the erythrocyte membrane and proteins could further point out the usefulness of R_m values in structure–activity studies.

Experimental Section

Materials and Methods. The steroids listed in Table I were obtained from drug companies or commercial sources. They can be divided into three groups deriving from 5α-androstane, 5β-pregnane, or estrane, respectively.



R_m Values Determination. The chromatographic technique for steroids has already been described.[1] The stationary nonpolar phase was represented by a silica gel G layer impregnated with silicone DC 200 (350 cSt) from Applied Sciences Laboratories. The mobile polar phase, saturated with silicone oil, consisted of H_2O in various mixtures (v/v) with Me_2CO. The concentration of Me_2CO in the mobile phase ranged from 15 to 65%. For a given compound at each of the different acetone concentrations were carried out at least eight determinations of the R_m value. The variability among the experimental results was not different from that usually observed with the above chromatographic technique in this laboratory. In particular, the standard error of the means was quite close to that previously reported for penicillins.[6]

Hemolytic Activity Determination. The details of the procedure have been already described.[1] The test compounds in Me_2SO, MeOH, EtOH, or Me_2CO solutions were added in 1- to 10-μl amounts to 4 ml of an erythrocyte suspension. At least 6–8 determinations were carried out for each concentration. Two series of control tubes were prepared. The assessment of 100% hemolysis was obtained by adding 3.8 ml of distilled H_2O to 0.2 ml of erythrocyte suspension. The hemolytic activity of Me_2SO, MeOH, EtOH, or Me_2CO was checked by adding 1–10 μl of each solvent to the system. After a 3-hr incubation at 37° all the tubes were centrifuged and the optical densities of the supernatants measured at 540 mμ in the Bausch and Lomb colorimeter. The results were expressed as percent of total hemolysis caused by distilled H_2O by means of the formula

$$\frac{(\text{OD of sample} - \text{OD of solvent control}) \times 100}{\text{OD of distilled } H_2O \text{ control}}$$

Results

R_m Values at 45% and Partition Coefficients. The chromatography of the steroid compounds with increasing acetone concentrations in the mobile phase resulted in decreasing R_m values. In particular for each compound there was a range of linear relationship between R_m values and acetone concentration in the mobile phase. The test compounds could be divided into several groups according to the acetone concentrations necessary in order to obtain a suitable range of R_m values. In Figure 1 a few examples are reported. As a reference system the R_m values at 45% were chosen. The reason was essentially that a 45% acetone concentration in the mobile phase provided an R_m value in the range of maximum accuracy for most of the compounds.

Green et al.[9] and Soczewinski et al.[10] considered the range of maximum accuracy of the R_m values to be from −0.95 to 0.95, i.e., calculated from R_f values between 0.1 and 0.9. The R_m values at 45% for compounds **20, 60, 64, 71,** and **86** were calculated by taking advantage of the linear

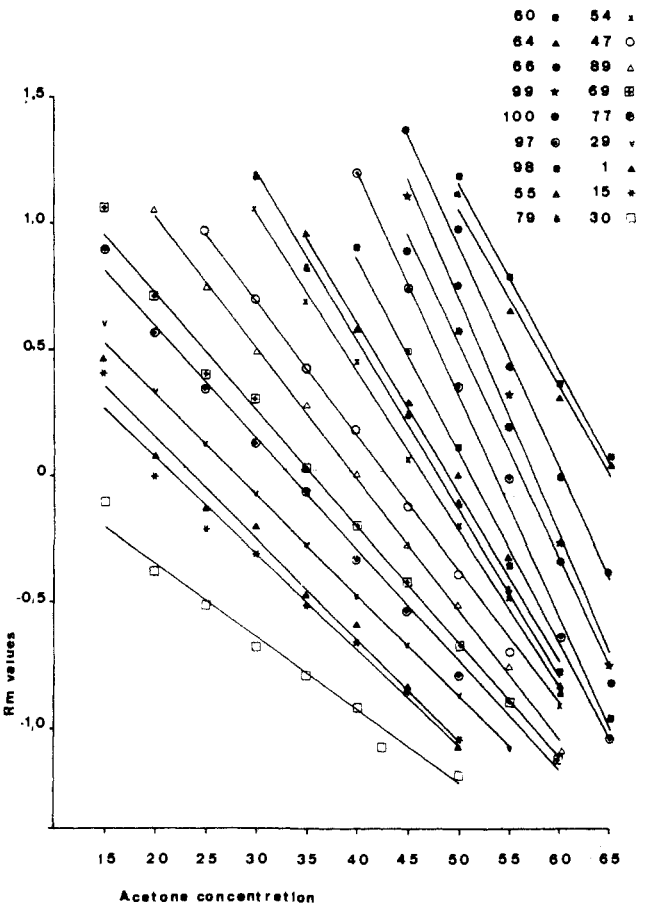


Figure 1. Relationship between R_m values and acetone concentration in the mobile phase. The compounds are numbered as in Table I.

relationship between experimental R_m values and acetone concentration in the mobile phase. In fact at 45% acetone for these compounds it was not possible to obtain a suitable migration.

The R_m values at 45% of Table I provide some understanding of the influence of substituent groups in determining the lipophilic character of the whole molecule.

The pregnane derivatives of Table I seem to be, as a group, less lipophilic than the androstane derivatives. The esterification of pregnane derivatives provided ΔR_m values lower than those obtained in the androstane and estradiol series. In the pregnane series the esterification at C_{21} provides ΔR_m values higher than those obtained at C_{17} (see hydroxyprogesterone and betamethasone esters). This is in agreement with Flynn,[4] who in a series of corticosteroids for the 17-acetate group found a π_e value lower than that for the 21-acetate one. The increasing of the R_m values from the lower to higher esters had been already shown.[1] Bowen et al.[11] found a similar increasing of the lipophilic character from the formate to valerate esters in series of androgen derivatives.

In Table II are reported the ΔR_m values for some substituent groups. The introduction of a hydroxy group lowers the R_m value. According to the position of the substituent OH group the hydrophilic effect increases in the following order: $17 \leq 11 < 21 < 16$. The introduction of a keto group lowers the R_m value; its substitution for an OH group does not change very much the lipophilic character. The introduction of a methyl group increases the R_m value. The effect is more evident with a 6α-CH_3 than with a 17α-CH_3. The substitution of a CH_3 group for an OH group has a similar influence. In particular, the difference between tri-

Table I. R_m, Log K_P, and π Values of Steroids[a]

Compd no.	Generic name	Chemical name	b	$a = R_m$	$R_{m(45\%)}$	Log K_P	π
1	Hydrocortisone	11β, 17α, 21-Trihydroxy-Δ^4-pregnene-3,20-dione	−0.040	0.96	−0.85	0.20	
2	Hydrocortisone 21-acetate	11β, 17α-Dihydroxy-Δ^4-pregnene-3,20-dione 21-acetate	−0.049	1.58	−0.58	1.42	
3	Hydrocortisone 21-butyrate	11β, 17α-Dihydroxy-Δ^4-pregnene-3,20-dione 21-butyrate		(2.22)	(−0.22)	2.38	
4	Hydrocortisone 21-caproate	11β, 17α-Dihydroxy-Δ^4-pregnene-3,20-dione 21-caproate		(3.19)	(0.17)	3.56	
5	Hydrocortisone 21-diethylaminoacetate	11β, 17α-Dihydroxy-Δ^4-pregnene-3,-20-dione 21-diethylaminoacetate	−0.045	2.06	0.02		
6	9α-Fluorohydrocortisone	9α-Fluoro-11β, 17α, 21-trihydroxy-Δ^4-pregnene-3,20-dione		(0.73)	(−0.82)	0.37	
7	9α-Fluorohydrocortisone 21-acetate	9α-Fluoro-11β, 17α-dihydroxy-Δ^4-pregnene-3,20-dione 21-acetate	−0.045	1.45	−0.54	1.66	
8	Corticosterone	11β, 21-Dihydroxy-Δ^4-pregnene-3,20-dione	−0.042	1.27	−0.66	0.66	
9	Corticosterone 21-acetate	11β-Hydroxy-Δ^4-pregnene-3,20-dione 21-acetate	−0.052	1.92	−0.41	(1.92)	
10	Deoxycorticosterone	21-Hydroxy-Δ^4-pregnene-3,20-dione	−0.049	1.78	−0.44	1.72	
11	Deoxycortisterone 21-acetate	21-Hydroxy-Δ^4-pregnene-3,20-dione 21-acetate	−0.059	2.49	−0.14	(2.98)	
12	Deoxycorticosterone trimethylacetate	21-Hydroxy-Δ^4-pregnene-3,20-dione 21-trimethylacetate	−0.060	2.56	−0.15		
13	Cortisone	17α, 21-Dihydroxy-Δ^4-pregnene-3,11,-20-trione	−0.038	0.88	−0.83	0.15	
14	Cortisone 21-acetate	17α-Hydroxy-Δ^4-pregnene-3,11,20-trione 21-acetate		(1.61)	(−0.55)	1.40	
15	Prednisone	17α, 21-Dihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione	−0.038	0.84	−0.86	(0.01)	
16	Prednisone 21-acetate	17α-Hydroxy-$\Delta^{1,4}$-pregnadiene-3,11,-20-trione 21-acetate	−0.052	1.77	−0.52	(1.28)	
17	6α-Chloroprednisone acetate	6α-Chloro-17α-hydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-acetate	−0.052	1.91	−0.42		
18	Prednisolone	11β, 17α, 21-Trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.040	0.90	−0.90	0.05	
19	Prednisolone 21-acetate	11β, 17α-Dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate	−0.048	1.54	−0.64	1.32	
20	Prednisolone palmitate	11β, 17α-Dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-palmitate	−0.148	9.15	2.49*		
21	9α-Fluoroprednisolone	9α-Fluoro-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.048	1.53	−0.69	(0.16)	
22	9α-Fluoroprednisolone 21-acetate	9α-Fluoro-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate		(2.27)	(−0.41)	(1.42)	
23	9α-Fluoro-6α-methylprednisolone	9α-Fluoro-6α-methyl-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione		(1.96)	(−0.51)	0.62	
24	9α-Fluoro-6α-methylprednisolone 21-acetate	9α-Fluoro-6α-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate		(2.70)	(−0.23)	1.92	
25	6α-Fluoroprednisolone	6α-Fluoro-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.040	0.95	−0.84	0.29	
26	6α-Fluoroprednisolone 21-acetate	6α-Fluoro-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate	−0.048	1.57	−0.62	1.57	
27	6α-Methylprednisolone	6α-Methyl-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.045	1.32	−0.72	0.54	
28	6α-Methylprednisolone 21-acetate	6α-Methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate	−0.055	1.98	−0.45	1.83	
29	11-Dehydrocorticosterone	21-Hydroxy-Δ^4-pregnene-3,11,20-trione	−0.040	1.14	−0.68		
30	Triamcinolone	9α-Fluoro-11β, 16α, 17α, 21-tetrahydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.029	0.24	−1.08	−0.12	
31	Triamcinolone 21-acetate	9α-Fluoro-11β, 16α, 17α-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate	−0.047	1.40	−0.76		

Table I *(Continued)*

Compd no.	Generic name	Chemical name	b	$a = R_m$	$R_{m(45\%)}$	Log K_p	π
31 (bis)	Triamcinolone acetonide	9α-Fluoro-11β, 21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 16α, 17α-acetonide		(1.63)	(−0.53)	1.17	
32	6α-Methyltriamcinolone acetonide	9α-Fluoro-6α-methyl-11β, 21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 16α, 17α-acetonide		(2.06)	(−0.35)	1.54	
33	6α-Methyltriamcinolone 21-acetate	9α-Fluoro-6α-methyl-11β-hydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 16α,-17α-acetonide 21-acetate		(2.80)	(−0.07)	2.93	
34	Fluocinolone acetonide	6α, 9α-Difluoro-11β, 21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 16α,-17α-acetonide	−0.051	1.69	−0.60	1.41	
35	Fluocinolone acetonide 21-acetate	6α, 9α-Difluoro-11-β-hydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 16α,-17α-acetonide 21-acetate	−0.060	2.49	−0.11	(2.67)	
36	Dexamethasone	9α-Fluoro-16α-methyl-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.046	1.30	−0.78	0.59	
37	Dexamethasone 21-acetate	9α-Fluoro-16α-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$pregnadiene-3,20-dione 21-acetate	−0.055	1.99	−0.47	(1.85)	
38	6α-Fluorodexamethasone	6α, 9α-Difluoro-16α-methyl-11β,-17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione		(1.36)	(−0.72)	0.87	
39	6α-Fluorodexamethasone 21-acetate	6α, 9α-Difluoro-16α-methyl-11β,-17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate		(2.04)	(−0.44)	2.16	
40	6α-Fluorodexamethasone 21-butyrate	6α, 9α-Difluoro-16α-methyl-11β,-17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-butyrate		(2.63)	(−0.09)	3.19	
41	Betamethasone	9α-Fluoro-16β-methyl-11β, 17α, 21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione	−0.045	1.26	−0.80	0.68	
42	Betamethasone 21-acetate	9α-Fluoro-16β-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-acetate	−0.054	1.89	−0.56	(1.94)	
43	Betamethasone 21-butyrate	9α-Fluoro-16β-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-butyrate	−0.061	2.52	−0.17	(2.86)	
44	Betamethasone 17-valerate	9α-Fluoro-16β-methyl-11β, 21-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 17α-valerate	−0.065	2.77	−0.15	2.71	
45	Betamethasone 21-caproate	9α-Fluoro-16β-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-caproate	−0.073	3.49	0.22	(4.03)	
46	Betamethasone 21-pelargonate	9α-Fluoro-16β-methyl-11β, 17α-dihydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione 21-pelargonate	−0.096	5.14	0.81		
47	Progesterone	Δ^4-Pregnene-3,20-dione	−0.053	2.28	−0.12	2.78	
48	17α-Hydroxyprogesterone	17α-Hydroxy-Δ^4-pregnene-3,20-dione	−0.053	2.00	−0.39		
49	17α-Hydroxyprogesterone 17-acetate	Δ^4-Pregnene-3,20-dione 17α-acetate	−0.055	2.26	−0.20		
50	17α-Hydroxyprogesterone 17-caproate	Δ^4-Pregnene-3,20-dione 17α-caproate	−0.074	3.77	0.41		
51	6α-Methyl-17α-hydroxyprogesterone acetate	6α-Methyl-Δ^4-pregnene-3, 20-dione 17α-acetate	−0.061	2.66	−0.09		
52		$\Delta^{4,16}$-Pregnadiene-3,20-dione	−0.055	2.30	−0.13		
53	Testosterone	17β-Hydroxy-Δ^4-androsten-3-one	−0.048	1.70	−0.45	1.94	−1.16
54	Testosterone 17-acetate	Δ^4-Androsten-3-one 17β-acetate	−0.063	2.93	0.07		−0.27
55	Testosterone 17-propionate	Δ^4-Androsten-3-one 17β-propionate	−0.068	3.34	0.29		0.23
56	Testosterone 17-isobutyrate	Δ^4-Androsten-3-one 17β-isobutyrate	−0.067	3.52	0.47		0.53

Table I *(Continued)*

Compd no.	Generic name	Chemical name	b	$a = R_m$	$R_{m(45\%)}$	Log K_p	π
57	Testosterone 17-valerate	Δ^4-Androsten-3-one 17β-valerate	−0.073	3.95	0.66		1.23
58	Testosterone 17-isocaproate	Δ^4-Androsten-3-one 17β-isocaproate	−0.080	4.50	0.91		1.53
59	Testosterone 17-enanthate	Δ^4-Androsten-3-one 17β-enanthate	−0.084	4.88	1.06		2.23
60	Testosterone 17-caprinate	Δ^4-Androsten-3-one 17β-caprinate	−0.075	5.93	1.53*		3.73
61	Testosterone 17-cypionate	Δ^4-Androsten-3-one 17β-cypionate	−0.092	5.32	1.14		2.37
62	Testosterone 17-phenylpropionate	Δ^4-Androsten-3-one 17β-phenylpropionate	−0.083	4.43	0.70		2.36
63	Testosterone 17-hexahydrobenzoate	Δ^4-Androsten-3-one 17β-hexahydrobenzoate	−0.082	4.61	0.85		1.74
64	Testosterone 17-undecylenate	Δ^4-Androsten-3-one 17β-undecylenate	−0.071	5.60	1.41*		3.93
65	Testosterone 17-benzoate	Δ^4-Androsten-3-one 17β-benzoate	−0.084	4.39	0.61		
66	Testosterone 17-caprinoylacetate	Δ^4-Androsten-3-one 17β-caprinoylacetate	−0.089	5.37	1.36		
67	Testosterone 17-butylphenoxyacetate	Δ^4-Androsten-3-one 17β-butylphenoxyacetate	−0.100	5.66	1.08		
68		Δ^4-Androstene-3β, 17β-diol	−0.050	1.74	−0.51		
69	Androstenedione	Δ^4-Androstene-3,17-dione	−0.046	1.64	−0.42		
70	Androstenediol	Δ^5-Androstene-3β, 17β-diol	−0.052	1.85	−0.49		
71	Androstenediol dipropionate	Δ^5-Androstene 3,17-dipropionate	−0.102	6.06	1.47*		
72		11β-Hydroxy-Δ^4-androstene-3,17-dione	−0.039	1.07	−0.68		
73	Norethindrone	17α-Ethynyl-17β-hydroxy-19-nor-Δ^4-androsten-3-one	−0.049	1.73	−0.53		
74	Norethindrone 17-acetate	17α-Ethynyl-19-nor-Δ^4-androsten-3-one 17β-acetate	−0.061	2.64	−0.13		
75	Fluoxymesterone	9α-Fluoro-17α-methyl-11β, 17β-dihydroxy-Δ^4-androsten-3-one	−0.046	1.43	−0.57		
76	17α-Methyltestosterone	17α-Methyl-17β-hydroxy-Δ^4-androsten-3-one	−0.051	1.92	−0.37		
77	Nandrolone	17β-Hydroxy-19-nor-Δ^4-androsten-3-one	−0.044	1.47	−0.53		
78	Nandrolone 17-propionate	19-Nor-Δ^4-androsten-3-one 17β-propionate	−0.060	2.90	0.22		
79	4-Chloronortestosterone 17-acetate	4-Chloro-19-nor-Δ^4-androsten-3-one 17β-acetate	−0.067	3.22	0.24		
80	4-Chlorotestosterone 17-acetate	4-Chloro-Δ^4-androsten-3-one 17β-acetate	−0.066	3.25	0.33		
81	Adrenosterone	Δ^4-Androstene-3,11,17-trione	−0.038	1.10	−0.65		
82	Norethandrolone	17α-Ethyl-17β-hydroxy-19-nor-Δ^1-androsten-3-one	−0.052	2.10	−0.29		
83	Dehydroepiandrosterone	3β-Hydroxy-Δ^5-androsten-17-one	−0.049	1.83	−0.41		
84	Methandriol	17α-Methyl-3β, 17β-dihydroxy-Δ^5-androstene	−0.054	2.06	−0.39		
85	Methandriol propionate	17α-Methyl-3β-hydroxy-Δ^5-androstene 17β-propionate	−0.073	3.83	0.55		
86	Methandriol dipropionate	17α-Methyl-Δ^5-androstene 3β, 17β-dipropionate	−0.104	6.29	1.61[b]		
87		$\Delta^{1,4}$-Androstadiene-3,17-dione	−0.042	1.35	−0.55		
88		Δ^1-5α-Androstene-3,17-dione	−0.049	1.82	−0.38		
89		17α-Methyl-17β-hydroxy-Δ^1-5α-androsten-3-one	−0.052	2.07	−0.28		
90	Epiandrosterone	3β-Hydroxy-5α-androstan-17-one	−0.050	1.96	−0.31		
91	Stanolone	17β-Hydroxy-5α-androstan-3-one	−0.051	2.01	−0 27		
92	Androstanedione	5α-Androstane-3, 17-dione	−0.049	1.95	−0.28		
93	Androsterone	3α-Hydroxy-5α-androstan-17-one	−0.057	2.42	−0.12		
94	Estradiol	3,17β-Estradiol	−0.051	1.85	−0.50		
95	Estradiol 17-valerate	3,17β-Estradiol 17β-valerate	−0.089	4.76	0.74		
96	Estradiol 17-enanthate	3,17β-Estradiol 17β-enanthate	−0.095	5.46	1.14		

Table I (Continued)

Compd no.	Generic name	Chemical name	b	$a = R_m$	$R_{m(45\%)}$	Log K_P	π
97	Estradiol 17-phenylpropionate	3,17β-Estradiol 17β-phenylpropionate	−0.089	4.77	0.74		
98	Estradiol 17-benzoate	3,17β-Estradiol 17β-benzoate	−0.077	3.94	0.49		
99	Estradiol 17-cypionate	3,17β-Estradiol 17β-cypionate	−0.094	5.40	1.11		
100	Estradiol dipropionate	3,17β-Estradiol 3β,17β-dipropionate	−0.086	4.83	0.89		
101	Ethynylestradiol	17α-Ethynyl-3,17β-estradiol	−0.060	2.27	−0.40		

[a] The calculated R_m or log K_P values are reported in parentheses. [b] For compounds **20, 60, 64, 71,** and **86,** see text.

amcinolone and dexamethasone again shows the strong hydrophilic effect of a 16α-OH group. On the other hand, a nor compound is less lipophilic than the parent compound, as in testosterone vs. nandrolone. The introduction of an ethyl, ethynyl, or fluoro group still increases the lipophilic character. The results are in agreement with those of Flynn.[8]

In order to establish the validity of the R_m values as an expression of the lipophilic character, their relationship with the log K_P values obtained by Flynn[8] in an ether–water system was studied (Table I). Equation 1 shows a

$$\log K_P = 3.292\ (\pm 0.310) + 3.398\ (\pm 0.459)\ R_{m(45\%)} \qquad n = 19,\ r = 0.968,\ s = 0.225 \qquad (1)$$

good correlation between R_m and log K_P values of 19 compounds.

However, by taking advantage of the additive property of the lipophilic character, the R_m or log K_P values for several more compounds could be calculated and are reported in Table I in parentheses.

The log K_P values were obtained by means of Flynn's π_e values. The log K_P value of prednisone was obtained from that of prednisolone.

$$\log K_P(\text{prednisolone}) - \underset{0.053}{\pi_e}(C_{11}\text{-hydroxy}) = \underset{0.043}{\log K_P(\text{prednisone})}$$

$$0.010$$

The log K_P value of 9α-fluoroprednisolone was obtained in the following way.

$$\log K_P(9\alpha\text{-fluoro-}6\alpha\text{-methylprednisolone}) - \underset{0.62}{\pi_e}(6\alpha\text{-methyl}) = \underset{0.16}{\log K_P}(9\alpha\text{-fluoroprednisolone})$$

$$0.46$$

The log K_P values of the 21-acetate derivatives of deoxycorticosterone, corticosterone, prednisone, 9α-fluoroprednisolone, dexamethasone, betamethasone, and fluocinolone acetonide were obtained by adding a π_e value of 1.265 to the log K_P value of the parent compound, as in the following examples.

$$\log K_P(\text{deoxycorticosterone}) + \underset{1.716}{\pi_e}(21\text{-acetate}) = \underset{1.265}{\log K_P}(\text{deoxycorticosterone acetate})$$

$$2.981$$

$$\log K_P(\text{corticosterone}) + \underset{0.655}{\pi_e}(21\text{-acetate}) = \underset{1.265}{\log K_P}(\text{corticosterone acetate})$$

$$1.920$$

$$\log K_P(\text{prednisone}) + \underset{0.010}{\pi_e}(21\text{-acetate}) = \underset{1.265}{\log K_P}(\text{prednisone acetate})$$

$$1.275$$

Similarly, the log K_P values of the butyrate and caproate esters of hydrocortisone were used in order to calculate the log K_P values of the same esters of betamethasone.

The R_m values of both parent compounds and 21-acetate esters were used in order to calculate the ΔR_m value of the 21-acetate group. In this way it was possible to calculate the R_m values of the acetate esters of cortisone, 9α-fluoroprednisolone, 9α-fluoro-6α-methylprednisolone, etc. At the same time by subtracting from the 9α-fluorohydrocortisone acetate, the R_m value of 9α-fluorohydrocortisone was obtained. The R_m values of butyrate and caproate esters of hydrocortisone and 6α-fluorodexamethasone were calculated by taking advantage of the R_m value of the corresponding betamethasone esters. The R_m values of 6α-fluorodexamethasone and 6α-methyltriamcinolone acetonide were calculated with the ΔR_m values of the 6α-fluoro and 6α-methyl groups from prednisolone derivatives. By adding or substracting the ΔR_m value of the 6α-methyl group, the R_m value of 9α-fluoro-6α-methylprednisolone or triamcinolone acetonide was respectively obtained.

In this way it was possible to calculate eq 2, which shows

$$\log K_P = 3.203\ (\pm 0.179) + 3.412\ (\pm 0.317)\ R_{m(45\%)} \qquad n = 42,\ r = 0.956,\ s = 0.300 \qquad (2)$$

a good relationship between R_m and Flynn's log K_P values.

In order to further establish the validity of the present R_m values, the Hansch π values of testosterone esters (Table I), as reported in an earlier paper,[1] were used in order to calculate eq 3.

$$\pi = -0.342\ (\pm 0.452) + 2.611\ (\pm 0.503)\ R_{m(45\%)} \qquad n = 12,\ r = 0.963,\ s = 0.424 \qquad (3)$$

This can be compared with eq 4, which had been previously calculated with the R_m values obtained at 54% acetone in the mobile phase.

The increase in the slope from eq 3 to eq 4 could be ex-

$$\pi = 0.581\ (\pm 0.287) + 3.281\ (\pm 0.566)\ R_{m(54\%)} \qquad n = 14,\ r = 0.963,\ s = 0.403 \qquad (4)$$

Table II. Influence of Substituent Groups on the Lipophilic Character of Steroids[a]

Group	Position	Compounds	$\Delta R_{m(45\%)}$	ΔR_m
OH	21	Progesterone vs. deoxycorticosterone	−0.32	−0.50
	17α	Progesterone vs. 17α-hydroxyprogesterone	−0.27	−0.28
	17α	Corticosterone vs. hydrocortisone	−0.19	−0.31
	17α	Corticosterone acetate vs. hydrocortisone acetate	−0.17	−0.34
	11β	Deoxycorticosterone vs. corticosterone	−0.22	−0.51
	11β	Deoxycorticosterone acetate vs. corticosterone acetate	−0.27	−0.57
	17α	11-Dehydrocorticosterone vs. cortisone	−0.15	−0.26
	16α	9α-Fluoroprednisolone vs. triamcinolone	−0.39	−1.29
	11β	Androstenedione vs. 11β-hydroxy-Δ^4-androstene-3,17-dione	−0.26	−0.57
Keto	11	Androstenedione vs. adrenosterone	−0.23	−0.54
	11	Deoxycorticosterone vs. dehydrocorticosterone	−0.24	−0.64
Double bond	Δ^4 vs. $\Delta^{1,4}$	Cortisone vs. prednisone	−0.03	−0.04
	Δ^4 vs. $\Delta^{1,4}$	Hydrocortisone vs. prednisolone	−0.05	−0.06
	Δ^4 vs. $\Delta^{1,4}$	Hydrocortisone acetate vs. prednisolone acetate	−0.06	−0.04
	Δ^4 vs. $\Delta^{1,4}$	Androstenedione vs. $\Delta^{1,4}$-androstadiene-3,17-dione	−0.13	−0.29
	Δ^4 vs. $\Delta^{4,16}$	Progesterone vs. $\Delta^{4,16}$-pregnadiene-3,-20-dione	−0.01	0.02
	Δ^4	Androstanedione vs. androstenedione	−0.14	−0.31
	Δ^4	Stanolone vs. testosterone	−0.18	−0.31
Keto vs. OH	11	Hydrocortisone vs. cortisone	0.02	−0.08
	11	Corticosterone vs. 11-dehydrocorticosterone	−0.02	−0.13
	11	Prednisolone vs. prednisone	0.04	−0.06
	11	Prednisolone acetate vs. prednisone acetate	0.12	0.23
	11	11β-Hydroxy-Δ^4-androstene-3,17-dione vs. adrenosterone	0.03	0.03
	3	Epiandrosterone vs. androstanedione	0.03	−0.01
Methyl	6α	Prednisolone vs. 6α-methylprednisolone	0.18	0.42
	6α	Prednisolone acetate vs. 6α-methylprednisolone acetate	0.19	0.44
	17α	Testosterone vs. 17α-methyltestosterone	0.08	0.22
	17α	Androstenediol vs. 17α-Methyl-Δ^5-androstene-3β, 17β-diol	0.10	0.21
Ethyl	17α	Nandrolone vs. norethandrolone	0.24	0.63
Ethynyl	17α	Nandrolone vs. norethindrone	0.00	0.26
	17α	Estradiol vs. ethynylestradiol	0.10	0.42
Fluoro	9α	Prednisolone vs. 9α-fluoroprednisolone	0.21	0.63
	9α	Hydrocortisone acetate vs. 9α-fluorohydrocortisone acetate	0.04	−0.13
	6α	Prednisolone vs. 6α-fluoroprednisolone	0.06	0.05
	6α	Prednisolone acetate vs. 6α-fluoroprednisolone acetate	0.02	0.03
Methyl vs. OH	16α	Triamcinolone vs. dexamethasone	0.30	1.06
Nor	19	Testosterone vs. nandrolone	−0.08	−0.23

[a] The ΔR_m and $\Delta R_{m(45\%)}$ values were obtained from the R_m and $R_{m(45\%)}$ values of Table I.

plained, according to Hansch[12] and Leo et al.,[13] with the change in the composition of the mobile phase. In a reversed-phase TLC system any alteration of the aqueous mobile phase to make it more like the nonaqueous one would increase the slope of the equation, where R_m is the independent variable.

The data of Table III can further contribute to the study of the relationship between partition coefficients in different systems. Scheuplein[14] and Scheuplein et al.,[15] as reported by Yotsuyanagi,[16] investigated the partitioning of a series of steroids between water and stratum corneum (K_e), amyl caproate (K_{ac}), or hexadecane (K_{hex}). Weber et al.[17] determined the partition coefficient of steroids in a column chromatography system by means both of elution curve (P_{1ec}) and solvent extraction (P_{1se}). The solvent system was prepared by shaking the solvents mixed in the appropriate proportions (*n*-hexane, 90; $CHCl_3$, 10; dioxane, 40; water, 5) and allowing the phases to separate. The lower

Table III. Partition Coefficients of Steroids in Various Systems[a]

	Log K_e	Log K_{ac}	Log K_{hex}	Log P_{lec}	Log P_{lse}
Prednisolone				1.73	1.66
6α-Fluoro-dexamethasone				1.55	1.52
Betamethasone				1.51	1.47
Prednisone				1.48	1.45
Hydrocortisone	0.84	0.11	-2.05	1.43	1.42
Paramethasone				1.41	1.37
Cortisone	0.93	0.18	-0.55	1.33	1.25
Fluocinolone acetonide				1.08	1.06
Triamcinolone acetonide				0.99	0.98
Prednisolone acetate				0.77	0.77
Hydrocortisone acetate				0.51	0.53
6α-Fluorodex-amethasone acetate				0.47	0.46
Paramethasone acetate				0.47	0.46
Betamethasone valerate				-0.12	-0.13
Progesterone	2.02	1.75	1.23		-0.51
Hydroxypro-gesterone	1.60	1.66	0.40		
Testosterone	1.36	1.20	0.41		
Corticosterone	1.23	0.83	-1.62		
Estradiol	1.66	1.82	-0.20		
Deoxycortico-sterone	1.57	1.48	0.48		

[a] For the calculation of eq 8 and 9, the R_m value at 45% paramethasone (-0.93) was calculated by substituting the ΔR_m value for the 6α-fluoro group to that for the 9α-fluoro group in compound 36 (Table I). The R_m value of its acetate was obtained by adding the R_m value for the 21-acetate group (+0.28).

phase was the aqueous phase; the upper phase was the organic one. By means of the R_m values of Tables I and III, eq 5-9 were calculated.

The correlation coefficient is fairly good in eq 5. Since

$$\log K_e = 0.238\ (\pm 0.205) + 1.581\ (\pm 0.359)\ R_{m(45\%)} \quad n = 8,\ r = 0.964,\ s = 0.094 \tag{5}$$

$$\log K_{ac} = 2.472\ (\pm 0.734) + 2.537\ (\pm 1.274)\ R_{m(45\%)} \quad n = 8,\ r = 0.887,\ s = 0.333 \tag{6}$$

$$\log K_{hex} = 1.916\ (\pm 1.282) + 4.063\ (\pm 2.226)\ R_{m(45\%)} \quad n = 8,\ r = 0.873,\ s = 0.582 \tag{7}$$

$$\log P_{lec} = -0.511\ (\pm 0.514) - 2.296\ (\pm 0.729)\ R_{m(45\%)} \quad n = 14,\ r = 0.891,\ s = 0.256 \tag{8}$$

$$\log P_{lse} = -0.620\ (\pm 0.380) - 2.403\ (\pm 0.559)\ R_{m(45\%)} \quad n = 15,\ r = 0.928,\ s = 0.242 \tag{9}$$

the log K_e values indicate the partitioning between stratum corneum and water, the R_m values could be used in studying the penetration of drugs through the skin.

Extrapolated R_m Values. The intercept of the equations of the straight lines describing the relationship between R_m values and acetone concentrations was used as the extrapolated R_m value at 0% acetone in the mobile phase. In Table I the intercepts of the mentioned equations are reported as R_m values. A very good correlation between extrapolated R_m and log K_P values is shown in eq 10 for the same 19 compounds used in calculating eq 1.

$$\log K_P = -0.979\ (\pm 0.422) + 1.459\ (\pm 0.274)\ R_m \quad n = 19,\ r = 0.937,\ s = 0.311 \tag{10}$$

By means of the ΔR_m of the extrapolated R_m values other R_m values were obtained and are reported in Table I in parentheses. The calculation was carried out in the same way as described for R_m values at 45% acetone. Equation 11 was calculated similarly to eq 2 with the data of 42 compounds. It is interesting to note that the R_m values used in calculating eq 11 range from 0.235 to 3.490, i.e., a range not very different from that of the log K_P values (from -0.121 to 4.032).

The decrease of the slope from eq 1 to eq 10 and from eq 2 to eq 11 could be explained according to Leo et al.[13] with

$$\log K_P = -0.982\ (\pm 0.353) + 1.402\ (\pm 0.183)\ R_m \quad n = 42,\ r = 0.923,\ s = 0.409 \tag{11}$$

the change in the composition of the mobile phase. Because of the good correlation coefficient of eq 10 and 11 one could have obtained similarly good correlations by using the extrapolated R_m values in order to calculate eq 3 and the subsequent eq 5-9. Since the extrapolation to 0% acetone in the mobile phase provides R_m values in a standard system for penicillins,[6] cephalosporins,[7] and phenols,[5] it could be very important to show that the extrapolation is valid also in the case of compounds much more differing in their lipophilic character. This would be clearly possible if the straight lines describing the relationship between experimental R_m values and acetone concentration were parallel. In Figure 1 the deviation from parallelism is evident for many compounds. This could explain the lower correlation coefficient of eq 10 and 11 when compared with eq 1 and 2. Moreover, at higher acetone concentration the partitioning should be different from that taking place at the lower concentrations. On the other hand, eq 10 and 11 could just show that the extrapolation provides partition data in fairly close relation with those at 45% acetone.

Biological Activity. In Table IV are reported the results of the hemolysis experiments. The hemolytic activity is expressed as log $1/C$ where C is the molar concentration of each compound which gives a 25% hemolysis (dosewise approach). The log $1/C$ values were obtained from the regression equations describing the linear relationship between log dose and hemolytic effect. A "t" test showed the high statistical significance of such equations. In the presence of steroids characterized by a very low lytic activity one could study, instead of hemolysis, their binding to the erythrocyte membrane. In fact, while the hemolytic activity seems to be the consequence of the interaction of higher concentrations of steroids with the erythrocyte membrane, at lower concentrations there should be a binding of the compounds to the membrane without any disrupting effect. In other words at lower concentrations one can determine the partitioning of a compound between the liquid medium and the erythrocyte membrane. This type of study was carried out in a previous paper.[4] The data on the binding of a series of steroids to the erythrocytes membrane are reported in Table IV as log BR values. In this way it was possible to take into consideration also compounds which in-

Table IV. Biological Activity Data[a]

Hemolytic act.			Membrane binding			Protein binding			Testosterone esters act.		
	Log 1/C			Log BR			Log BR			Log BR	
Compd	Obsd	Calcd (eq 15)	Compd	Obsd	Calcd (eq 21)	Compd	Obsd	Calcd (eq 24)	Compd	Obsd	Calcd (eq 25)
98	4.097	3.968	53	1.02	0.89	72	1.60	1.47	53	0.00	0.04
94	3.715	3.547	54	1.06	1.37	18	1.69	1.57	54	0.12	0.23
100	4.097	3.938	55	1.44	1.49	1	1.49	1.54	55	0.25	0.29
44	4.000	3.817	56	1.66	1.55	13	1.50	1.58	56	0.43	0.32
99	4.097	3.841	57	1.77	1.66	69	1.28	1.14	56b	0.39	0.32
20	2.222	2.013	58	1.88	1.77	8	1.25	1.35	57	0.48	0.39
86	2.970	3.618	59	1.92	1.84	53	1.08	1.11	60	0.61	0.69
47	3.699	3.690	60	1.82	1.84	83	0.95	1.03			
71	3.523	3.690	61	1.92	1.90	10	0.90	1.06			
61	4.152	3.868	62	1.92	1.76	47	0.81	0.77			
50	3.678	3.960	64	1.77	1.93	93	0.74	0.68			
79	4.046	3.900	66	1.95	1.90						
66	3.523	3.858	69	0.94	0.86						
36	3.097	3.321	76	0.98	0.99						
27	3.721	3.330	77	0.90	0.78						
1	3.334	3.158	78	1.22	1.36						
30	2.304	2.752	82	0.90	1.06						

[a] The table includes all compounds which gave measurable results in terms of hemolytic activity and membrane binding. [b] The R_m value of isobutyrate esters was used in the case of the butyrate derivatives.

teract with the erythrocyte membrane at an extent lower than that necessary for causing hemolysis. Obviously one could have obtained an hemolytic effect with these compounds also, if it would have been possible to have them in the system at very high concentrations. In Table IV are finally reported some data on the protein binding of steroids and the biological activity of testosterone esters. Part of these data have been used in correlation studies with R_m values.[2,3]

Structure–Activity Relationship. The relationship between the R_m values and the log 1/C values of Table IV describing the hemolytic activity is provided by eq 12–15. The introduction of the R_m^2 term into eq 12 and 14 improves the correlation coefficient in a significant way.

$$\log 1/C = 3.556\ (\pm 0.343) - 0.028\ (\pm 0.317)\ R_{m(45\%)} \quad n = 17,\ r = 0.033,\ s = 0.617 \quad (12)$$

$$\log 1/C = 3.933\ (\pm 0.166) + 0.516\ (\pm 0.143)\ R_{m(45\%)} - 0.514\ (\pm 0.098)\ R_m^{2}{}_{(45\%)} \quad n = 17,\ r = 0.898,\ s = 0.281 \quad (13)$$

$$\log 1/C = 3.616\ (\pm 0.592) - 0.019\ (\pm 0.134)\ R_m \quad n = 17,\ r = 0.039,\ s = 0.614 \quad (14)$$

$$\log 1/C = 2.598\ (\pm 2.503) + 0.659\ (\pm 0.231)\ R_m - 0.079\ (\pm 0.019)\ R_m^2 \quad n = 17,\ r = 0.869,\ s = 0.316 \quad (15)$$

In a previous work[1] the hemolytic activity of a series of testosterone esters was shown to be parabolically related with the R_m values at 54% acetone in the mobile phase. Those hemolysis data as well as the present R_m values were used in order to calculate eq 16 and 17.

A comparison of eq 17 with eq 15 shows that a larger number of compounds did not substantially change the character of the relationship (Figure 2).

$$\log BR = 0.960\ (\pm 0.205) + 1.867\ (\pm 0.472)\ R_{m(45\%)} - 1.011\ (\pm 0.355)\ R_m^{2}{}_{(45\%)} \quad n = 12,\ r = 0.949,\ s = 0.192 \quad (16)$$

$$\log BR = -2.923\ (\pm 1.354) + 2.043\ (\pm 0.719)\ R_m - 0.220\ (\pm 0.088)\ R_m^2 \quad n = 12,\ r = 0.934,\ s = 0.219 \quad (17)$$

As shown by eq 19 and 21 calculated with the log BR values of Table IV, a parabolic relationship is also present between lipophilic character and membrane binding of steroids. The calculated log BR values of Table IV were obtained from eq 21.

$$\log BR = 1.219\ (\pm 0.098) + 0.561\ (\pm 0.119)\ R_{m(45\%)} \quad n = 17,\ r = 0.927,\ s = 0.161 \quad (18)$$

$$\log BR = 1.280\ (\pm 0.097) + 0.782\ (\pm 0.201)\ R_{m(45\%)} - 0.239\ (\pm 0.188)\ R_m^{2}{}_{(45\%)} \quad n = 17,\ r = 0.951,\ s = 0.137 \quad (19)$$

$$\log BR = 0.527\ (\pm 0.215) + 0.262\ (\pm 0.055)\ R_m \quad n = 17,\ r = 0.930,\ s = 0.161 \quad (20)$$

$$\log BR = -0.001\ (\pm 0.478) + 0.606\ (\pm 0.298)\ R_m - 0.047\ (\pm 0.038)\ R_m^2 \quad n = 17,\ r = 0.950,\ s = 0.134 \quad (21)$$

The data of Table IV regarding the protein binding of corticosteroids and androgens and the duration of action of testosterone esters allowed the calculation of eq 22 and 23. Here the introduction of the R_m^2 term did not improve the correlation coefficient in a significant way.

By substituting in eq 22 and 23 the R_m values at 45% with the extrapolated R_m values, eq 24 and 25 were obtained.

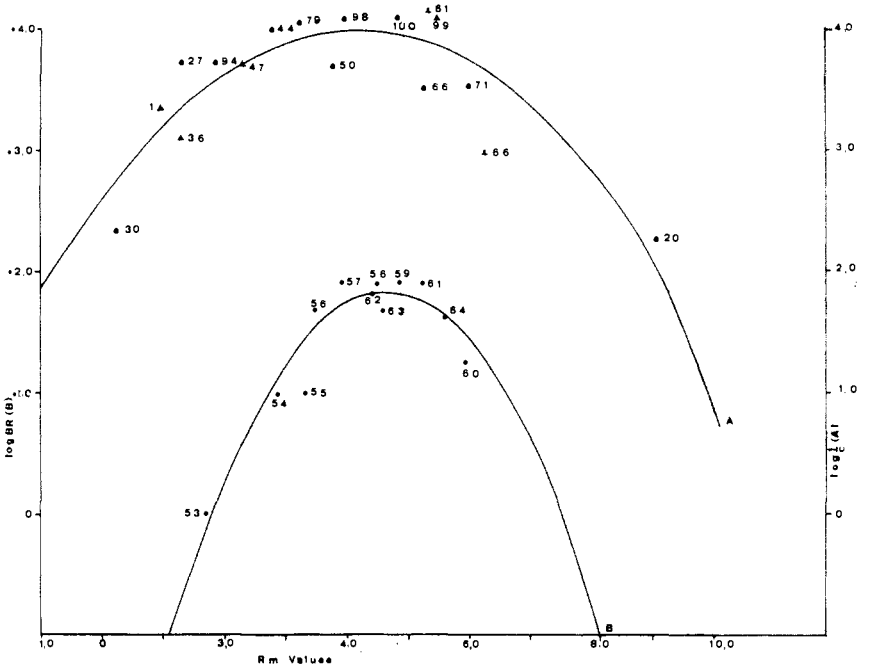


Figure 2. Relationship between R_m values and hemolytic activity. The log BR values of compounds 53–64 (●) were taken from ref 1a. The log $1/C$ values of compounds 1, 20, 27, 30, 36, 44, 47, 50, 61, 66, 71, 79, 86, 94, 98, and 100 (▲) are reported in Table IV. The compounds are numbered as in Table I. Curves A and B were obtained by means of eq 15 and 17.

$$\log \text{BR (protein binding steroids)} = 0.608\ (\pm 0.212) - 1.125\ (\pm 0.359)\ R_{m(45\%)} \quad (22)$$

n 11, r 0.918, s 0.137

$$\log \text{BR (duration of action of testosterone esters)} = 0.182\ (\pm 0.092) + 0.331\ (\pm 0.133)\ R_{m(45\%)} \quad (23)$$

7, 0.939, 0.077

The slope of eq 24 is close to those of equations describing the relationship between lipophilic character and protein binding.[12] On the other hand, the lower slope of eq 25

$$\log \text{BR} = 2.094\ (\pm 0.217) - 0.583\ (\pm 0.135)\ R_m \quad (24)$$

(n 11, r 0.952, s 0.104)

$$\log \text{BR} = -0.215\ (\pm 0.287) + 0.152\ (\pm 0.077)\ R_m \quad (25)$$

(n 7, r 0.906, s 0.094)

seems to be close to those of equations describing the structure–activity relationship in more complex systems.[12]

Conclusion

The lipophilic character of steroids seems to play an important role in different biological systems. Its importance in regulating their hemolytic activity and binding to erythrocyte membrane is particularly evident. Segal et al.[18] pointed out that the esterification of an OH group or its oxidation to the respective ketone always enhances hemolysis. However, in the present results the substitution of an OH group for the respective ketone would seem to provoke a decrease in the lipophilic character and therefore a decrease in hemolytic activity rather than an increase.

Florence et al.[19] suggested that the adsorption of steroids at lipid surfaces and their penetration into lipid membranes could be involved in the alleged increased platelet aggregation in patients taking oral contraceptive steroids over long periods of time. They supported this hypothesis with the interaction of steroids with lipid monolayers and erythrocyte membrane.[19] The stabilization–lysis action of antiinflammatory steroids on lysosomes,[20] or more generally on membranes, represents another important factor of the action of steroids. The in vivo activity of long-acting steroid esters could be explained with a slower absorption from the site of administration or with a protection from metabolism until they reach their site of action.[2] The protein binding of steroids seems to be an important factor in regulating their distribution in the whole organism and therefore their walk to the site of action and/or availability for biological activity.[21,22] In particular, Scholtan[23] pointed out the influence of the hydrophobic character of steroids on their protein binding. The above outline of aspects of the biological activity of steroids shows the importance of the lipophilic character. Obviously this does not rule out the possibility of more specific requirements for the drug–receptor interaction. An optimal lipophilic character of steroids could be necessary in order to make the drug available for such interaction.

James[24] and James et al.[25] analyzing our previous work on the time of maximal effect in fowl of a series of testosterone esters found a better relationship between R_m values and biological activity when determining their R_m values in the Bush system of petroleum ether–methanol–formic acid. This would point out the usefulness of further investigations in order to find better models of the lipid–water distribution of drugs in biological systems.

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Regression Analysis of the Relationship between Physical Properties and the in Vitro Inhibition of Monoamine Oxidase by Propynylamines

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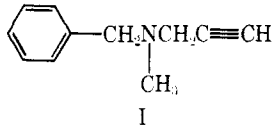
Regression analysis of the potency of inhibition of monoamine oxidase by 47 propynylamines revealed that there are three determinants of inhibitory potency: (1) the smallest substituent on the nitrogen must be methyl or hydrogen in order for any activity to be observed; (2) potency is parabolically related to pK_a—the optimum pK_a is 6.2; and (3) ortho-substituted benzylamine analogs are ten times more potent than predicted on the basis of pK_a values. The optimum pK_a cannot be explained by differences in fraction ionized but rather in terms of the multistep sequence whereby these compounds inhibit MAO. A very slight positive effect of hydrophobicity on potency was found. The potency of several analogs not included in the original analysis was predicted.

The monoamine oxidase, MAO [amine:oxygen oxidoreductase (deaminating), E.C. 1.4.3.4], inhibitor pargyline (I) is by now a well-known drug.[1] At the time of its discovery a number of analogs were prepared and tested. A preliminary discussion of the structure and activity of some of these compounds has also been reported.[2] At the time of the initial evaluation of the analogs, we also measured their pK_a's. Variations in pK_a do not in themselves explain differences in potency, and at the time of the original studies in 1960 we were not able to discern any relationship between physical properties and potency. The introduction of multiple regression analysis to the study of quantitative structure–activity relationships of drugs provided the necessary tool for an examination of our data.[3]



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There is considerable evidence that pargyline produces its irreversible inhibition of MAO in a several-step sequence. Since pargyline is a competitive inhibitor of MAO when it is added to the enzyme simultaneously with the substrate, the first step in the inhibition is postulated to be a reversible substrate-like binding.[4] In the second step, the MAO oxidizes pargyline by abstracting a proton from one of the methylene groups.[5] Finally, the modified form of the pargyline reacts to form a covalent bond to the enzyme with the result that the enzymatic activity is lost.[4]

One of the objectives of our quantitative structure–activity analysis was to explore the possibility that the relative potency of various analogs which react in such a complex scheme would be correlated with physical properties. If such a correlation were found, would it be consistent with the proposed mode of inhibition of MAO by pargyline? Could the relative potency of the various inhibitors have been predicted by the relative binding constants and maximal velocities of substrates?

Methods. pI_{50}. The approximate pI_{50} value (negative log of the molar concentration which inhibits MAO 50%) was determined as previously described.[2] The reactions were conducted in 10-ml beakers in a Dubnoff shaker. In brief, graded concentrations of the inhibitor were preincubated 30 min at 38° in 0.1 *M* phosphate buffer, pH 7.2, with rat liver mitochondria. The mitochondria has been isolated by differential centrifugation and stored at −5°. After the preincubation, serotonin creatinine phosphate was added to produce a final concentration of 0.01 *M*. The reaction of MAO with the serotonin proceeded for 2.5 hr. By the end of this incubation period the contents of the control beakers had turned dark brown due to reactions of the aldehyde formed in the deamination of serotonin. In contrast, the contents of vessels in which the MAO was completely inhibited were the same off-white color as before the addition of substrate. For evaluation, the contents of the beakers were poured into small test tubes. The color