THE APPLICATION OF PAPER CHROMATOGRAPHY TO THE STUDY OF STEROID STRUCTURE

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INTRODUCTION

Analysis of the urine from newborn infants by a paper chromatographic method[1] has revealed the presence of a large number of compounds with the properties of neutral steroids which are not prominent in the urine of adults[2].

All but a few of these occur in quantities so limited that their isolation, in pure form and in reasonable amount, for purposes of identification by all the standard macrotechniques, is extremely difficult. However, much useful information may be obtained by the direct application of chemical tests to small quantities of steroids on paper chromatograms, and the value of these tests is immeasurably increased if there is concomitant systematic application of the mathematical theory of chromatography.

MARTIN's[3] basic theory of a set mathematical relationship between the partition coefficient of a compound and its chemical structure led to the introduction by BATE-SMITH AND WESTALL[4] of the useful term R_M, where R_M equals log $(1/R_F-1)$ and when information concerning the structure of a compound is to be derived from paper chromatographic work R_M values are employed rather than R_F values since they are more directly related to the different chemical groups contained in the molecule. In the case of the steroids, evidence that the relationships between R_M values are more nearly constant than those between R_F values can be found in the results of REINEKE[5], NEHER[6], KABASAKALIAN AND BASCH[7] and BUSH[8].

In a chromatographic system, and even in any one chromatography tank, reproducible R_F values are only achieved if there is absolute stability of a number of factors[9], and the BUSH systems, which require prolonged equilibration of solvents and paper, may present problems in the maintenance of tank equilibrium. They are also markedly influenced by changes in temperature[7]. The present analysis of the mean R_F and R_M values obtained by the repeated chromatography of standard steroids in BUSH systems under strictly controlled conditions has been undertaken in an attempt to check the accuracy of the results in chromatographic systems which are being utilized in the identification of unknown steroid compounds.

The difference between two R_M values is the ΔR_M and BUSH[8] has used the term ΔR_{Mg} for any ΔR_M value due to the substitution of another group for a hydrogen atom. He has defined ΔR_{Mr} as any ΔR_M value due to a change of molecular structure other than the simple substitution of a hydrogen atom. These terms and others advocated by BUSH[8] have been adopted for the presentation of this report.

CHROMATOGRAPHIC SYSTEMS

Bush L/85: Light petroleum (b.p. 100–120°)–methanol–water (100:85:15, by vol.). Temperature 23°. Equilibration 15 h.

Bush LB21/80: Light petroleum (b.p. 100–120°)–benzene–methanol–water (67:33:80:20, by vol.). Temperature 28°. Equilibration 5 h.

Bush T/75: Toluene–methanol–water (100:75:25, by vol.). Temperature 28°. Equilibration 5 h.

Bush LB21/A85: Light petroleum (b.p. 100–120°)–benzene–glacial acetic acid–water (67:33:85:15, by vol.). Temperature 28°. Equilibration 3 h.

In all cases, strips of Whatman No. 42 paper (50 × 2.5 cm) have been used. All paper has been washed as suggested by BUSH AND WILLOUGHBY[10].

CONTROL OF CONDITIONS

All chromatography tanks are screened from draughts in rooms with thermostatic control at 23° and 28° ± 0.5°. A constant circulation of air throughout the rooms is maintained by fans. Only a few square feet of the internal walls of the chromatography rooms are in structural contact with the external walls of the building and the areas concerned have been lined with insulating material.

The internal milieu of the tanks is satisfactorily maintained by paper lining the ends of the tanks dipping into the mobile phase, while a curtain of lint suspended from a glass rod or wire stretching along the length of the centre of the tank dips into the stationary phase contained in conical flasks in the bottom of the tank. The solvent front can thereby be seen readily on all papers in the tank. Each time tanks are loaded the presence of sufficient stationary phase is checked and the beakers are replenished only with stationary phase which has recently been prepared and equilibrated. In the case of solvent front runs a measured volume of mobile phase is added for each run. When only a few sample papers are to be run in a tank, resolution is improved by including blank strips.

The use of dyestuffs in chromatography, as recommended by NEHER, MEYSTRE AND WETTSTEIN[11] and by BUSH[8] has proved invaluable in obtaining accurate measurements of R_F values.

RESULTS

The mean R_F values for a large number of steroids and for dyes developed in the systems L/85, LB21/80, T/75 and LB21/A85, obtained from a series of runs, are given in Tables I to IV.

The R_F values are given corrected to three decimal places since, in most instances, each is the mean of a number of results. In any single run an R_F value cannot be measured to this degree of accuracy, but R_F values have not been corrected to two decimal places in the present report since the mean values obtained by arithmetic have been used in the calculation of the R_M values. ΔR_M values in these systems are presented in Tables V to X.

TABLE I

MEAN R_F AND R_M VALUES FOR STEROIDS AND A DYE IN BUSH SYSTEM L/85
Whatman No. 42 paper; Temperature 23°.

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
P^4-11β-ol-3,20-one	11β-OH-progesterone	2	0.04	1.3802
αA-3α-ol-11,17-one	11-oxoandrosterone	1	0.04	1.3802
A^4-3,11,17-one	adrenosterone	53	0.06	1.1965
P^4-17α-ol-3,20-one	17α-OH-progesterone	2	0.08	1.0607
A^4-17β-ol-3-one	testosterone	4	0.10	0.9542
A^4-17α-ol-3-one	*cis*-testosterone	4	0.13	0.8255
αA-3β-ol-17-one	epiandrosterone	1	0.17	0.6886
A^5-3β-ol-17-one	DHA	63	0.19	0.6269
βA-3α-ol-17-one	aetiocholanolone	62	0.245	0.4888
A^4-3,17-one	androstenedione	12	0.31	0.3475
αA-3α-ol-17-one	androsterone	21	0.34	0.2880
P^5-3β-ol-20-one	pregnenolone	2	0.39	0.1942
αA-3,17-one	5α-androstanedione	1	0.50	0.0
P^4-3,20-one	progesterone	3	0.56	—0.1046
βP-3,20-one	pregnanedione	8	0.72	—0.4101
	Waxoline purple	21	0.80	—
αA-17-one	5α-androstan-17-one	2	0.87	—0.8268

TABLE II

MEAN R_F AND R_M VALUES FOR STEROIDS AND DYES IN BUSH SYSTEM LB21/80
Whatman No. 42 paper; Temperature 28°.

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
P^4-17α,21-ol-3,11,20-one	cortisone E	4	0.005	2.3077
βP-17α,21-ol-3,11,20-one	dihydrocortisone DHE	4	0.017	1.7753
P^4-6β,21-ol-3,20-one	6β-OH-DOC	4	0.026	1.5805
P^4-11β,17α-ol-3,20-one	21-deoxy F	9	0.030	1.5096
P^4-11β,21-ol-3,20-one	corticosterone B	23	0.037	1.4142
A^4-19-ol-3,17-dione	19-OH-androstenedione	1	0.040	1.3802
βP-3α,17α-ol-11,20-one		4	0.045	1.3267
P^4-17α,21-ol-3,20-one	compound S	7	0.056	1.2269
P^4-21-ol-3,11,20-one	Kendall's compound A	10	0.073	1.1038
A^5-3β-ol-7,17-one	7-oxodehydroepiandrosterone	6	0.079	1.0663
	Lacquer violet	96	0.081	—
βA-3α,11β-ol-17-one	11β-OH-aetiocholanolone	78	0.104	0.9353
P^4-16α-ol-3,20-one	16α-OH-progesterone	2	0.108	0.9170
P^4-17α,21-ol-3,11,20-one(-21-OAc)	cortisone-21-acetate	1	0.122	0.8572
αP-11β,21-ol-3,20-one	allodihydro B	13	0.138	0.7956
αA-3α,11β-ol-17-one	11β-OH-androsterone	75	0.161	0.7169
A^4-11β-ol-3,17-one	11β-OH-androst-4-ene-3,17-dione	75	0.195	0.6157
βA-3α-ol-11,17-one	11-oxoaetiocholanolone	81	0.252	0.4725
βP-17α,21-ol-3,11,20-one(-21-OAc)	dihydro E 21-acetate	1	0.254	0.4679
P^4-6β-ol-3,20-one	6β-OH-progesterone	9	0.276	0.4188
P^5-3β,17α-ol-20-one	17α-OH-pregnenolone	1	0.292	0.3847
P^4-11β-ol-3,20-one	11β-OH-progesterone	10	0.300	0.3680
αA-3α-ol-11,17-one	11-oxoandrosterone	79	0.308	0.3516

(*continued on p.* 365)

TABLE II (*continued*)

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
P^5-3β,21-ol-20-one	21-OH-pregnenolone	28	0.313	0.3414
βP-3α,17α-ol-20-one	17α-OH-pregnanolone	40	0.344	0.2804
P^4-11β,21-ol-3,20-one(-21-OAc)	corticosterone-21-acetate	1	0.349	0.2707
A^4-3,11,17-one	adrenosterone	92	0.395	0.1853
βP-3α,21-ol-20-one	tetrahydro DOC	5	0.411	0.1562
A^4-17β-ol-3-one	testosterone	49	0.424	0.1329
P^4-21-ol-3,11,20-one(-21-OAc)	compound A-21-acetate	1	0.425	0.1313
P^4-17α-ol-3,20-one	17α-OH-progesterone	15	0.432	0.1189
P^4-21-ol-3,20-one	DOC	25	0.478	0.0382
A^4-17α-ol-3-one	*cis*-testosterone	14	0.482	0.0315
	Neher dye F_{11}	52	0.495	—
αA-3,11,17-one	5α-androstanetrione	7	0.550	—0.0872
A^5-3β-ol-17-one	dehydroepiandrosterone	86	0.571	—0.1244
P^4-20α-ol-3-one		3	0.602	—0.1798
βA-3α-ol-17-one	aetiocholanolone	16	0.610	—0.1945
αA-3α-ol-17-one	androsterone	16	0.694	—0.3556
βP-21-ol-3,20-one	dihydro DOC	3	0.710	—0.3893
A^4-3,17-one	androst-4-ene-3,17-dione	7	0.776	—0.5391
P^5-3β-ol-20-one	pregn-5-enolone	2	0.778	—0.5452
αA-3,17-one	5α-androstanedione	8	0.828	—0.6819
P^4-3,20-one	progesterone	10	0.844	—0.7328
P^5-3β,17α-ol-20-one(-3-OAc)	17α-OH-pregnenolone-3-acetate	1	0.857	—0.7773
βA-3α-ol-11,17-one(-3-OAc)	11-oxoaetiocholanolone-3-acetate	2	0.866	—0.8097
P^4-6β-ol-3,20-one(-6-OAc)	6β-OH-progesterone-6-acetate	1	0.882	—0.8729
αA-17-one	5α-androstan-17-one	8	0.917	—1.0410
	Waxoline purple	59	0.919	—

TABLE III

MEAN R_F AND R_M VALUES FOR STEROIDS AND DYES IN BUSH SYSTEM T/75

Whatman No. 42 paper; Temperature 28°.

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
P^4-6β,11β,17α,21-ol-3,20-one	6β-OH F	4	0.002	2.6981
P^4-6β,17α,21-ol-3,11,20-one	6β-OH E	48	0.016	1.7889
P^4-11β,17α,20α,21-ol-3-one		1	0.029	1.5247
P^4-11β,17α,20β,21-ol-3-one		3	0.042	1.3581
βP-3α,11β,17α,21-ol-20-one	THF	48	0.067	1.1440
αP-3α,11β,17α,21-ol-20-one	allo-THF	9	0.089	1.0103
αP-3β,17α,21-ol-11,20-one	3β-allo-THE	3	0.096	0.9741
βP-3α,17α,21-ol-11,20-one	THE	52	0.113	0.8949
P^4-11β,17α,21-ol-3,20-one	cortisol, F	130	0.156	0.7339
	Neher dye F_{14}	10	0.207	—
P^4-11β,21-ol-18-al-3,20-one	aldosterone	3	0.232	0.5211
βP-11β,17α,21-ol-3,20-one	dihydrocortisol	8	0.283	0.4041
P^4-17α,21-ol-3,11,20-one	cortisone, E	107	0.290	0.3888
αP-3β,11β,21-ol-20-one	3β-allo-THB	3	0.364	0.2430
βP-3α,11β,21-ol-20-one	THB	8	0.410	0.1580
P^4-6β,21-ol-3,20-one	6β-OH DOC	4	0.424	0.1329
βP-17α,21-ol-3,11,20-one	dihydrocortisone	17	0.426	0.1300

(*continued on p.* 366)

TABLE III (*continued*)

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
αP-17α,21-ol-3,11,20-one	allodihydrocortisone	2	0.430	0.1225
βP-3α,17α,21-ol-20-one	THS	4	0.434	0.1152
βP-3α,17α-ol-11,20-one		2	0.532	—0.0555
A^5-3β,16α-ol-17-one	16α-OH DHA	2	0.534	—0.0590
βP-3α,21-ol-11,20-one	THA	8	0.536	—0.0625
P^4-11β,21-ol-3,20-one	corticosterone	30	0.606	—0.1864
	Lacquer violet	6	0.608	—
P^4-17α,21-ol-3,20-one	compound S	5	0.618	—0.2083
A^5-3β-ol-7,17-one	7-oxo-DHA	3	0.620	—0.2125
βA-3α,11β-ol-17-one	11β-OH-aetiocholanolone	1	0.638	—0.2464
	Neher dye F_5	11	0.669	—
P^4-21-ol-3,11,20-one	compound A	45	0.692	—0.3516
A^4-11β-ol-3,17-one	11β-OH-androstenedione	2	0.750	—0.4776
βA-3α-ol-11,17-one	11-oxoaetiocholanolone	2	0.797	—0.5935
A^4-17β-ol-3-one	testosterone	2	0.839	—0.7167
A^4-17α-ol-3-one	*cis*-testosterone	2	0.850	—0.7545
A^4-3,11,17-one	adrenosterone	2	0.869	—0.8210
	Neher dye F_{11}	4	0.869	—
A^5-3β-ol-17-one	DHA	3	0.878	—0.8570
P^4-21-ol-3,20-one	deoxycorticosterone	12	0.882	—0.8729
βA-3α-ol-17-one	aetiocholanolone	1	0.884	—0.8827
αA-3α-ol-17-one	androsterone	1	0.891	—0.9136
A^4-3,17-one	androstenedione	3	0.903	—0.9706
αA-3,17-one	5α-androstane-3,17-dione	2	0.927	—1.1024
	Waxoline purple	41	0.936	—
αA-3-one	5α-androstanone	1	0.939	—1.1871

TABLE IV

MEAN R_F AND R_M VALUES FOR STEROIDS AND DYES IN BUSH SYSTEM LB21/A85
Whatman No. 42 paper; Temperature 28°.

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
P^4-6β,17α,21-ol-3,11,20-one	6β-OH-E	1	0.001	2.9203
P^4-11β,17α,21-ol-3,20-one	F	1	0.012	1.9193
P^4-17α,21-ol-3,11,20-one	E	2	0.015	1.8263
βP-3α,11β,17α,21-ol-20-one	THF	2	0.0235	1.6185
βP-3α,17α,21-ol-11,20-one	THE	2	0.024	1.6093
βP-11β,17α,21-ol-3,20-one	dihydro F	1	0.033	1.4642
βP-17α,21-ol-3,11,20-one	dihydro E	1	0.036	1.4240
P^4-6β,21-ol-3,20-one	6β-OH-DOC	1	0.042	1.3549
P^4-17α,21-ol-3,11,20-one(-21-OAc)	E-21-acetate	2	0.051	1.2669
P^4-11β,17α-ol-3,20-one	21-deoxy F	1	0.053	1.2504
P^4-21-ol-3,11,20-one	A	2	0.055	1.2342
	Neher dye F_5	8	0.063	—
P^4-11β,21-ol-3,20-one	B	2	0.066	1.1516
αP-3β,11β,21-ol-20-one	3β-allo-THB	1	0.077	1.0799
P^4-16α-ol-3,20-one	16α-OH-progesterone	2	0.0855	1.0294
A^5-3β-ol-7,17-one	7-oxo-DHA	4	0.086	1.0257
βP-3α,11β,21-ol-20-one	THB	2	0.089	1.0111

(*continued on p.* 367)

TABLE IV (*continued*)

Compound	*Trivial name*	*No. of estimations*	R_F	R_M
	Lacquer violet	35	0.106	—
A4-11β-ol-3,17-one	11β-OH-androstenedione	4	0.110	0.9080
P4-21-ol-3,11,20-one(-21-OAc)	A-21-acetate	1	0.149	0.7567
P4-6β-ol-3,20-one	6β-OH-progesterone	1	0.153	0.7432
P4-11β,21-ol-3,20-one(-21-OAc)	B-21-acetate	1	0.156	0.7332
P4-11β-ol-3,20-one	11β-OH-progesterone	1	0.165	0.7043
βA-3α,11β-ol-17-one	11β-OH-aetiocholanolone	11	0.168	0.6948
P4-17α,21-ol-3,20-one(-21-OAc)	S-21-acetate	1	0.181	0.6556
A4-3,11,17-one	adrenosterone	13	0.183	0.6497
αA-3α,11β-ol-17-one	11β-OH-androsterone	13	0.193	0.6213
βA-3α-ol-11,17-one	11-oxoaetiocholanolone	14	0.248	0.4817
P4-21-ol-3,20-one	DOC	3	0.258	0.4588
αA-3α-ol-11,17-one	11-oxoandrosterone	9	0.265	0.4431
P4-17α-ol-3,20-one	17α-OH-progesterone	1	0.278	0.4145
P4-16α-ol-3,20-one(-16-OAc)	16α-OH-progesterone-16-acetate	1	0.286	0.3974
βP-3α,11β,17α,21-ol-20-one-(-3,21-OAc)	THF-3,21-diacetate	1	0.288	0.3931
P5-3β,21-ol-20-one	21-OH-pregn-5-enolone	7	0.301	0.3659
	Neher dye F_{11}	45	0.334	—
A4-17β-ol-3-one	testosterone	1	0.335	0.2978
A4-17α-ol-3-one	*cis*-testosterone	1	0.345	0.2786
βP-3α,21-ol-20-one	tetrahydro DOC	1	0.393	0.1889
A5-3β-ol-7,17-one(-3-OAc)	7-oxo-DHA-3-acetate	2	0.409	0.1599
A4-3,17-one	androst-4-ene-3,17-dione	4	0.421	0.1383
A5-3,17-one	androst-5-ene-3,17-dione	1	0.421	0.1383
P4-21-ol-3,20-one(-21-OAc)	DOC-21-acetate	1	0.461	0.0679
αA-3α,11β-ol-17-one(-3-OAc)	11β-OH-androsterone-3-acetate	7	0.465	0.0611
A5-3β-ol-17-one	DHA	8	0.469	0.0539
P5-3β,21-ol-20-one(-21-OAc)	21-OH-pregn-5-enolone-21-acetate	2	0.495	0.0086
βA-3α-ol-17-one	aetiocholanolone	8	0.522	−0.0381
βA-3α,11β-ol-17-one(-3-OAc)	11β-OH-aetiocholanolone-3-acetate	6	0.542	−0.0731
βP-3α,11β,21-ol-20-one(-3,21-OAc)	THB-3,21-diacetate	1	0.548	−0.0835
αA-3α-ol-11,17-one(-3-OAc)	11-oxoandrosterone-3-acetate	4	0.562	−0.1085
αA-3α-ol-17-one	androsterone	7	0.581	−0.1421
P4-3,20-one	progesterone	6	0.591	−0.1599
βA-3α-ol-11,17-one(-3-OAc)	11-oxoaetiocholanolone-3-acetate	9	0.608	−0.1904
αA-3,17-one	5α-androstane-3,17-dione	1	0.618	−0.2090
A5-3β-ol-17-one(-3-OAc)	DHA-3-acetate	2	0.777	−0.5421
βP-3α,21-ol-20-one(-3,21-OAc)	tetrahydro-DOC-3,21-diacetate	1	0.792	−0.5800
αA-3α-ol-17-one(-3-OAc)	androsterone-3-acetate	2	0.795	−0.5884
P5-3β,21-ol-20-one(-3,21-OAc)	21-OH-pregnenolone-3,21-diacetate	3	0.831	−0.6925
βA-3α-ol-17-one(-3-OAc)	aetiocholanolone-3-acetate	2	0.835	−0.7033
	Waxoline purple	58	0.858	—
αA-17-one	5α-androstan-17-one	1	0.870	−0.8268
	Sudan red	22	0.889	—

TABLE V

ΔR_{Mg} VALUES FOR HYDROXYLATION IN THE SYSTEM LB21/80
(Temperature 28°)

Substituent	*Root compound*	ΔR_{Mg}
6β-OH	P4-21-ol-3,20-one	+1.54
	P4-3,20-one	+1.15

(*continued on p.* 368)

TABLE V (*continued*)

Substituent	*Root compound*	ΔR_{Mg}
11β-OH	P^4-17α-ol-3,20-one	+1.39
	P^4-21-ol-3,20-one	+1.38
	βA-3α-ol-17-one	+1.13
	αA-3α-ol-17-one	+1.07
	A^4-3,17-one	+1.15
	P^4-3,20-one	+1.10
16α-OH	P^4-3,20-one	+1.65
17α-OH	P^4-21-ol-3,11,20-one	+1.20
	P^4-11β-ol-3,20-one	+1.14
	P^4-21-ol-3,20-one	+1.19
	P^5-3β-ol-20-one	+0.93
	P^4-3,20-one	+0.85
20α-OH	P^4-3,20-one	+0.55
20β-OH	P^4-3,20-one	+0.37
21-OH	P^4-11β-ol-3,20-one	+1.07
	P^4-17α-ol-3,20-one	+1.13
	P^5-3β-ol-20-one	+0.89
	P^4-3,20-one	+0.89

TABLE VI

ΔR_{Mg} VALUES FOR KETONE GROUPS IN THE SYSTEM LB21/80
(Temperature 28°)

Substituent	*Root compound*	ΔR_{Mg}
7-oxo	A^5-3β-ol-17-one	+1.13
11-oxo	P^4-17α,21-ol-3,20-one	+1.08
	P^4-21-ol-3,20-one	+1.07
	βA-3α-ol-17-one	+0.67
	αA-3α-ol-17-one	+0.71
	A^4-3,17-one	+0.72
	αA-3,17-one	+0.60

TABLE VII

ΔR_{Mr} VALUES FOR ACETYLATION IN THE SYSTEM LB21/80
(Temperature 25°)

Conversion	*Root compound*	ΔR_{Mr}
3α-OH ↓ 3α-OAc	βA-3α-ol-11,17-one	—1.28
3β-OH ↓ 3β-OAc	P^5-3β,17α-ol-20-one	—1.16
6β-OH ↓ 6β-OAc	P^4-6β-ol-3,20-one	—1.29

(*continued on p.* 369)

TABLE VII (*continued*)

Conversion	*Root compound*	ΔR_{Mr}
21-OH ↓ 21-OAc	P^4-17α,21-ol-3,11,20-one	—1.45
	βP-17α,21-ol-3,11,20-one	—1.31
	P^4-11β,21-ol-3,20-one	—1.14

TABLE VIII

ΔR_{Mg} VALUES IN THE SYSTEM T/75
(Temperature 28°)

Substituent	*Root compound*	ΔR_{Mg}
6β-OH	P^4-11β,17α,21-ol-3,20-one	+1.96
	P^4-17α,21-ol-3,11,20-one	+1.40
	P^4-21-ol-3,20-one	+1.01
11β-OH	βP-3α,17α,21-ol-20-one	+1.03
	P^4-17α,21-ol-3,20-one	+0.94
	P^4-21-ol-3,20-one	+0.69
	βA-3α-ol-17-one	+0.64
	A^4-3,17-one	+0.49
16α-OH	A^5-3β-ol-17-one	+0.80
17α-OH	βP-3α,11β,21-ol-20-one	+0.99
	βP-3α,21-ol-11,20-one	+0.96
	P^4-11β,21-ol-3,20-one	+0.92
	P^4-21-ol-3,11,20-one	+0.74
	P^4-21-ol-3,20-one	+0.66
20α-OH	P^4-11β,17α,21-ol-3,20-one	+0.79
20β-OH	P^4-11β,17α,21-ol-3,20-one	+0.62
21-OH	βP-3α,17α-ol-11,20-one	+0.95
7-oxo	A^5-3β-ol-17-one	+0.64
11-oxo	βP-3α,17α,21-ol-20-one	+0.77
	P^4-17α,21-ol-3,20-one	+0.60
	P^4-21-ol-3,20-one	+0.52

TABLE IX

ΔR_{Mg} VALUES IN THE SYSTEM LB21/A85
(Temperature 28°)

Substituent	*Root compound*	ΔR_{Mg}
6β-OH	P^4-17α,21-ol-3,11,20-one	+1.09
	P^4-21-ol-3,20-one	+0.90
	P^4-3,20-one	+0.90

(*continued on p.* 370)

TABLE IX (*continued*)

Substituent	*Root compound*	ΔR_{Mg}
11β-OH	P^{4}-21-ol-3,20-one	+0.69
	βP-3α,21-ol-20-one	+0.82
	A^{4}-3,17-one	+0.77
	P^{4}-21-ol-3,20-one(-21-OAc)	+0.67
	P^{4}-3,20-one	+0.86
	βA-3α-ol-17-one	+0.73
	αA-3α-ol-17-one	+0.76
16α-OH	P^{4}-3,20-one	+1.19
17α-OH	P^{4}-11β,21-ol-3,20-one	+0.77
	P^{4}-21-ol-3,11,20-one	+0.69
	βP-3α,11β,21-ol-20-one	+0.61
	P^{4}-11β-ol-3,20-one	+0.55
	P^{4}-21-ol-3,20-one(-21-OAc)	+0.59
	P^{4}-3,20-one	+0.57
21-OH	P^{4}-11β,17α-ol-3,20-one	+0.67
	P^{4}-6β-ol-3,20-one	+0.61
	P^{4}-3,20-one	+0.62
7-oxo	A^{5}-3β-ol-17-one	+0.97
11-oxo	P^{4}-21-ol-3,20-one	+0.78
	A^{4}-3,17-one	+0.51
	βA-3α-ol-17-one	+0.52
	αA-3α-ol-17-one	+0.59
	αA-17-one	+0.62

TABLE X

ΔR_{Mr} VALUES IN SYSTEMS LB21/80, LB21/A85, L/85 AND T/75
(Temperature 28°)

Conversion	*Root compound*	ΔR_{Mr} *in systems*			
		LB21/80	*LB21/A85*	*L/85*	*T/75*
5β(H)-3α-OH	βP-3α,17α-ol-20-one	+0.1	—	—	—
↓ Δ^{5}-3β-OH	βA-3α-ol-17-one	+0.07	+0.09	+0.14	+0.03
5β(H)-3α-OH	βP-3α,11β,17α,21-ol-20-one	—	+0.30	—	—0.41
↓	βP-3α,17α,21-ol-11,20-one	—	+0.22	—	—0.50
Δ^{4}-3-oxo	βP-3α,11β,21-ol-20-one	—	+0.14	—	—0.34
	βP-3α,17α,21-ol-20-one	—	—	—	—0.32
	βP-3α,21-ol-11,20-one	—	—	—	—0.29
5β(H)-3α-OH	βP-3α,17α,21-ol-11,20-one	—	—	—	+0.080
↓	βP-3α,11β,21-ol-20-one	—	+0.07	—	+0.085
5α(H)-3β-OH	βA-3α-ol-17-one	—	—	+0.20	—
5β(H)-3α-OH	βP-3α,11β,17α,21-ol-20-one	—	—	—	—0.13
↓	βA-3α,11β-ol-17-one	—0.22	—0.07	—	—
5α(H)-3α-OH	βA-3α-ol-11,17-one	—0.12	—0.04	—	—
	βA-3α-ol-17-one	—0.16	—0.10	—0.20	—0.03

DISCUSSION

R_F values of compounds have been obtained either from their position relative to that of the solvent front or from overruns in which standard compounds or dyes with known R_F values have been included. For example, in the LB21/80 system, the R_F values for adrenosterone, DOC, F_{11} and less polar compounds have been measured from runs in which the position of the solvent front has been ascertained. The R_F values of the more polar steroids have been obtained from overruns and based upon the R_F values of one or more of these reference compounds used as a marker. In the T/75 system, the R_F values of THB and compounds less polar have been calculated from runs in which the position of the solvent front has been located, while the R_F values of the more polar compounds have been calculated from overruns and based upon previously determined R_F values—that of dihydrocortisone for chromatograms which have been overrun for a limited period, and that of cortisol for chromatograms which have had prolonged overrunning.

A stable environment for chromatography is reflected in the ΔR_M values listed in Tables V to X. In all the systems it can be seen that one specific structural change in the molecule produces a change in the R_M value of a steroid compound which is very nearly constant.

In all cases, the steroids have been listed in the tables in descending order of polarity and this reveals that the ΔR_{Mg} values for oxy-groups tend to decrease as the polarity of the root compound decreases. However, the values obtained show that the ΔR_{Mg} value for a specific oxy-group is virtually constant for root substances of near polarity.

Apart from the isolated values given in previous reports from this laboratory[1,2], only a few R_F values for steroids run in these aqueous methanol systems have been published but several of the ΔR_M values obtained in the present study parallel those published by BUSH[8] for steroids run in closely related systems.

The results given here not only provide further evidence for the validity of the theory of BATE-SMITH AND WESTALL[4] but also show that chromatography is being carried out under suitably stable conditions. However, in practice, even with the strict attention to detail during chromatography which has been described, variations in R_F values do occur from one run to another. Of the systems studied, the LB21/80 and the L/85 are the most prone to show this and the possibility of errors arises in ΔR_M calculations based on the average R_F values obtained from a number of runs although the chances of serious errors are much less than in calculations based on the results from isolated runs.

In consequence, when unknown steroid compounds are submitted to chromatography during their structural investigation it is advisable to carry out a preliminary check of the agreement of the mobility of a suitable dyestuff relative to the solvent front with the average R_F value of that dye established over a period of time in the laboratory. Thereafter, unknown compounds and their derivatives may be run in the tank but it is essential to run them in association with appropriate standards and dyes. If the relationships between the R_M values of the standard compounds included in any such run are found to be consistent with those regularly achieved in the laboratory it is reasonable to assume that the average ΔR_M values established for the particular chromatographic system are applicable in considerations of the possible nature of the steroids under investigation.

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SUMMARY

Mean R_F and R_M values have been obtained for a number of steroids and dyes in BUSH aqueous methanol systems. Scrutiny of the ΔR_M values verifies the stability of the conditions achieved.

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