APPLICATION OF THIN-LAYER CHROMATOGRAPHY TO THE STEROIDS OF THE ANDROSTANE SERIES

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Thin-layer chromatography utilizing Silica gel G with a binder has been successfully employed for the separation of several classes of steroids, *e.g.* etianic acids[3], oestrogens[10], Δ^4-3-oxo-C_{21}-steroids[18] and 19-norsteroids[12].

For a number of steroids of the androstane series, thin-layer chromatography has been applied without a binder using alumina[6], or with a binder using silica gel-starch[27], silica gel G[11,23] or alumina G[23].

The variation in techniques employed and the small number of steroids studied, does not permit extensive application of these results, *e.g.* for ΔR_M-function calculation.

Our experience has been with more than 50 steroids of the androstane series, and this paper describes the application of nine solvent systems to twenty-nine steroids of this series, nearly all of them saturated, and the calculation of the function ΔR_{Mg} for any hydroxyl and ketogroup. Forthcoming communications will cover the Δ^4-3-oxo- and Δ^5-3-hydroxy-steroids of this series.

MATERIALS

Reagents

All reagents used were of analytical grade, and the solvents were redistilled through fractionating columns before use. Sulphuric acid (batch No. 731) glacial acetic acid (batch No. 60), acetic anhydride (batch 42) and *m*-dinitrobenzene (batch No. 3114) were obtained from Merck A.G., Darmstadt. Anisaldehyde(*p*-methoxybenzaldehyde) (S 5209), chromic acid anhydride (A 229), bismuth subnitrate (A 1878) and osmium tetroxide (3616) were from Kebo A.B., Stockholm. Pyridine was purchased from the Gas Works of Stockholn, potassium iodide (batch No. 3162) from Baker Chemical Co., Philipsburg, U.S.A. and potassium hydroxide from E.K.A. (Elektrokemiske A.B., Bohus), Sweden.

Steroids

The sources of the steroids used in this investigation are given in Table I. In addition to their systematic names, trivial names are given, whenever indicated. The abbreviations *ol* and *one* are used to designate one or more hydroxyl- and oxo-groups, respectively.

METHODS AND RESULTS

General methods

The method used in this investigation has been described in a previous paper[10]. In this series of experiments, one-dimensional chromatograms were run on Silica

TABLE I

SYSTEMATIC NAMES, TRIVIAL NAMES, ABBREVIATIONS, SOURCES AND COLOURS OBTAINED WITH ANISALDEHYDE-SULFURIC ACID REACTION FROM THE 29 STEROIDS STUDIED IN THIS EXPERIMENT

Systematic name	*Trivial name*	*Abbreviation*	*Source**	*Colour***
5α-Androstan-11-one	Androstan-11-one	5α A 11 one	(e)	No colour
5β-Androstan-11-one	Etiocholan-11-one	5β A 11 one	(e)	No colour
5α-Androstan-17-one	Androstan-17-one	5α A 17 one	(d)	Blue
5α-Androstane-3,17-dione	Androstanedione	5α A 3,17 one	(c)	Olive
5β-Androstane-3,17-dione	Etiocholanedione	5β A 3,17 one	(d)	Brown
5α-Androst-1-ene-3,17-dione	1-Dehydro-androstanedione	Δ^1 5α A 3,17 one	(d)	Violet blue
Androst-4-ene-3,17-dione	Androstenedione	Δ^4 A 3,17 one	(c)	Salmon
Estr-4-ene-3,17-dione	19-Norandrostenedione	19-nor Δ^4 A 3,17 one,	(d)	Orange-brown
5α-Androst-2-ene-7,17-dione		Δ^2 5α A 7,17 one	(a)	Olive
5β-Androstane-3,11,17-dione	11-Oxo-etiocholanolone	5β A 3,11,17 one	(e)	Red brown
5α-Androstan-17-ol		17β ol 5α A	(b)	Blue
17β-Hydroxy-5α-androstan-3-one	5α-Dihydrotestesterone	17β ol 5α A 3 one	(d)	Brown-olive to blackish-olive
17β-Hydroxy-5β-androstan-3-one	5β-Dihydrotestosterone	17β ol 5β A 3 one	(c)	Bright violet
17β-Hydroxy-androst-4-en-3-one	Testosterone	17β ol Δ^4 A 3 one	(c)	Red-orange to violet-purple
3α-Hydroxy-5α-androstan-17-one	Androsterone	3α ol 5α A 17 one	(c)	Green
3β-Hydroxy-5α-androstan-17-one	Epiandrosterone	3β ol 5α A 17 one	(c)	Green
3α-Hydroxy-5β-androstan-17-one	Etiocholanolone	3α ol 5β A 17 one	(c)	Green
3β-Hydroxy-5β-androstan-17-one	5β-Epiandrosterone	3β ol 5β A 17 one	(c)	Green
11β-Hydroxy-5α-androstane-3,17-dione	11β-Hydroxy-androstanedione	11β ol 5α A 3,17 one	(e)	Brown
17β-Hydroxy-5α-androstane-3,17-dione	7-Oxo-5α-dihydrotestosterone	17β ol 5α A 3,7 one	(a)	Brown
3α-Hydroxy-5β-androstane-11,17-dione	11-Oxo-etiocholanolone	3α ol 5β A 11,17 one	(e)	Brown-olive
3α-Hydroxy-5α-androstane-7,17-dione	7-Oxo-androsterone	3α ol 5α A 7,17 one	(a)	Green-olive
3β-Hydroxy-5α-androstane-7,17-dione	7-Oxo-epiandrosterone	3β ol 5α A 7,17 one	(a)	Green-olive
5α-Androstane-3α,17β-diol		3α,17β ol 5α A	(b)	Blue
5α-Androstane-3β,17β-diol		3β,17β ol 5α A	(c)	Blue
Androst-4-ene-3β,17β-diol		3β,17β ol Δ^4 A	(d)	Dark blue
5β-Androstane-3α,17β-diol		3α,17β ol 5β A	(d)	Blue
3β,11β-Dihydroxy-5α-androstan-17-one	11β-Hydroxy-epiandrosterone	3β,11β ol 5α A 17 one	(c)	Green
3α,11β-Dihydroxy-5β-androstan-17-one	11β-Hydroxy-etiocholanolone	3α,11β ol 5β A 17 one	(e)	Dark grey-blue

* Source (a) Dr. J. Joska, Academy of Sciences, Prague, Czechoslovakia; (b) Dipl. Ing. I. Könyves, Hälsingborg, Sweden; (c) A. G. Schering, Berlin, Germany; (d) Steraloids Inc., Flushing 52, N.Y., U.S.A.; (e) U.S.P. Steroid Reference Substance.

** Anisaldehyde-sulfuric acid reaction.

gel G (Merck A.G., Darmstad, batches No. 132307 and No. 62631). The plates were prepared, using the apparatus for thin-layer chromatography described by STAHL[28], from a mixture obtained by homogenizing the Silica gel for 4–5 min with 80 ml of distilled water; 45 g of batch No. 132307 or 30 g of batch No. 62631 were used. The plates were dried in a stream of hot air followed by heating for 30 min at 100–105°.

Every chromatogram was run in completely saturated tanks. By totally covering three of the walls and the fourth wall partially with a double layer of thick filter paper soaked in solvent, complete saturation was obtained. The chambers must be equilibrated for at least 4 h prior to each chromatogram. Reproducible R_F values could only be obtained by maintaining complete saturation of the chambers in this manner. Under these experimental conditions, comparable R_F values were obtained for chromatoplates kept in desiccators, over anhydrous silica gel (Blaugel, Silica Gel Ges. Hamburg, Germany), or exposed to the air for over 24 h.

During the introduction of the chromatoplate into the chamber great care must be taken. The plate is introduced into the chamber with the silica gel facing the wholly covered walls by raising, just as much as is necessary, that part of the cover of the chamber above the partially covered wall.

In spite of all these precautions, the upper layers of the chamber become unsaturated and this cannot be overcome during the short period of development. To avoid the influence of this unsaturation on the R_F values of extremely weakly polar steroids, the run should be terminated when the solvent front is 15 cm from the starting line. However, the calculated ΔR_{Mg} and ΔR_{Mr} values with an R_F over 0.75 are only approximations because of this effect. 1–25 μg of samples was dissolved in 5–15 μl of ethanol or methanol–chloroform (1:1) mixture using a 0.01 Blaubrand pipette (Colodur, Hamburg, Germany). The origin was 2.5–3 cm from the lower edge of the plate and at least 2 cm from the lateral border, to prevent variation in R_F values due to capillary action. The edges of the silica gel on the plate were marked in each case.

Detection of the steroids

For detection of the spots, the following reactions were used:

(a) *Anisaldehyde–sulphuric acid reaction*[9,21]. The plates were sprayed with a 1 % (v/v) solution of anisaldehyde in a 2 % (v/v) solution of concentrated sulphuric acid in glacial acetic acid. After spraying, the chromatoplates were heated to 95–100° for 12–15 min.

The colours developed are recorded in Table I.

(*b*) *The Zimmermann reaction*[32]. This reaction for methylene groups, activated by an oxo-group in *ortho* position, was used for the detection of 17- and 3-oxo-steroids. After being sprayed with a freshly prepared mixture of equal parts of a 2 % ethanolic solution of *m*-dinitrobenzene and 1.25 *N* ethanolic potassium hydroxide, the plates were exposed to a stream of hot air. 3-Oxosteroids appear immediately as blue spots while 17-oxo-steroids with an unsubstituted 16 position give the classical violet colour after 3 to 6 min.

(*c*) *Dragendorff's reagent*. This was suggested by PELCOVA[26] for steroids, especially α,β-unsaturated ketosteroids. It was prepared in the following modified manner: 10 ml of 0.3 % bismuth subnitrate solution in 50 % (v/v) sulphuric acid was added with constant agitation to 30 ml of 10 % KI in 70 % (v/v) ethanol. This reagent must be prepared every second day and kept at a low temperature (about $+4°$).

After spraying the plates with this reagent, the Δ^4-3-oxosteroids almost immediately developed an orange colour. This reaction is not specific, and a yellow or yellow-orange colour occurred with several steroids. It was primarily used here for the identification of etiocholan-11-one and androstan-11-one, which are difficult to identify by other methods.

(*d*) *Conversion of alcoholic steroids to ketosteroids, in situ.* The oxosteroid so formed was detected by the Zimmermann reaction (KUPFER *et al.*[17], PAN[24]). Plates were sprayed with a 0.25 % solution of chromic acid anhydride in glacial acetic acid and heated for 15 min at 90–95°. The plates were then subjected to the Zimmermann reaction as previously described.

(*e*) *Osmium tetroxide.* Double bonds were detected by the exposure of the plates to the vapour of osmium tetroxide under sealed conditions[10]. Isolated double bonds such as Δ^2-5-androstene-7,17-dione, form an osmate ester after 5 to 10 min exposure. Conjugated double bonds, as found in 1-dehydro-androstanedione and testosterone react after 20 to 40 min, and $\Delta^{1,4}$-conjugated bonds (17-hydroxy-$\Delta^{1,4}$-androstadien-3-one and $\Delta^{1,4}$-androstadiene-3,17-dione) after 1 h. Osmium tetroxide is a very sensitive reagent for 2-hydroxyoestrogens[19a] and reacts immediately with it.

Solvent systems and chromatographic results

(*a*) *Solvent systems and symbols.* In this study the following systems, which have been described previously[18,19] were employed:

System A: ethyl acetate 45 parts, cyclohexane 45 parts and abs. ethanol, 10 parts.
System C: ethyl acetate 50 parts, cyclohexane 50 parts.
System D: chloroform 90 parts, abs. ethanol 10 parts.
System E: ethyl acetate 72 parts, water saturated *n*-hexane 13.5 parts, abs. ethanol 4.5 parts and glacial acetic acid 10 parts.
System H: benzene 40 parts, abs. ethanol 10 parts.
System K: benzene 90 parts, abs. ethanol 10 parts.
System L: chloroform 19 parts, abs. ethanol 1 part.
System N: benzene 19 parts, abs. ethanol 1 part.

In addition, the following new systems were used:

System M: ethyl acetate 75 parts, *n*-hexane 20 parts, and acetic acid 5 parts.
System O: *n*-hexane 75 parts, ethyl acetate 25 parts.

With the exception of system O, developed for steroids of extremely high polarity R_F- and R_S-values (S = testosterone), the standard deviation S.D. and the function R_M of almost all the steroids studied have been calculated. However, values for androstan-11-one and etiocholan-11-one were only calculated in five systems: N, C, L, K and O.

The R_M-function was calculated according to the definition given by BATE-SMITH AND WESTALL[4]:

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

Symbols ΔR_{Mg}, ΔR_{Mr} and ΔR_{Ms} are employed here with the same meaning as that used by BUSH[5]. The ΔR_{Mg} of a radical is a ΔR_M resulting from the substitution of a hydrogen atom in the molecule by this radical. $\Delta R_{Mr} = \Delta R_{M1} - \Delta R_{M2}$ and is the ΔR_M resulting from the substitution of one radical for another, *e.g.*, by means of the reduction of an oxo group. The ΔR_M of a steroid in two solvent systems is called ΔR_{Ms}. The R_M concept is being employed here in adsorption systems although its general validity is usually only recognised for partition systems.

(*b*) *Chromatographic results.* The chromatographic results of the application of ten systems to twenty-nine steroids of the androstane series are summarised in Tables II to XI. It can be seen from these Tables that the separation of isomers which differ in the 5α-(androstane) and 5β-(etiocholane or testane) configuration, without substitution in ring A, is difficult and occasionally impossible. Androstan-11-one and etiocholan-11-one could not be separated in any of the systems, while the separation of androstanedione and etiocholanedione could only be achieved in systems K, L, N, M and O.

(*Text continued on p. 401*)

TABLE II

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY FIVE STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM A*. THIN LAYER CHROMATOGRAPHY CARRIED OUT UNDER COMPLETE SATURATION ON SILICA GEL G (MERCK A.G., DARMSTADT, BATCH NO. 132307)

Steroid	*n****	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	12	0.75	0.03	0.68–0.82	−0.477	1.52	0.03	1.45–1.59
Δ^2 5α A 7,17 one	8	0.67	0.02	0.62–0.72	−0.308	1.43	0.08	1.23–1.63
17β ol 5α A	10	0.64	0.03	0.58–0.70	−0.250	1.31	0.04	1.22–1.40
5β A 3,17 one	12	0.64	0.03	0.58–0.70	−0.250	1.29	0.06	1.17–1.41
5α A 3,17 one	9	0.63	0.03	0.57–0.69	−0.231	1.28	0.07	1.13–1.43
Δ^1 5α A 3,17 one	8	0.61	0.02	0.56–0.66	−0.194	1.30	0.03	1.10–1.50
3β ol 5β A 17 one	9	0.57	0.02	0.51–0.63	−0.122	1.16	0.04	1.08–1.24
17β ol 5α A 3 one	12	0.57	0.02	0.52–0.62	−0.122	1.16	0.03	1.10–1.22
11β ol 5α A 3,17 one	8	0.57	0.03	0.49–0.65	−0.122	1.15	0.10	0.82–1.38
3α ol 5α A 17 one	11	0.55	0.04	0.47–0.63	−0.087	1.19	0.04	1.11–1.27
17β ol 5β A 3 one	9	0.55	0.04	0.47–0.63	−0.087	1.12	0.08	0.93–1.31
3β ol 5α A 17 one	9	0.52	0.03	0.46–0.58	−0.035	1.06	0.03	1.00–1.12
5β A 3,11,17 one	7	0.52	0.04	0.43–0.61	−0.035	1.04	0.08	0.84–1.24
3α 17β ol 5α A	9	0.50	0.03	0.41–0.55	0.000	1.02	0.04	0.94–1.10
3β 17β ol Δ^4 A	12	0.50	0.03	0.43–0.57	0.000	1.00	0.05	0.89–1.11
3α ol 5β A 17 one	11	0.49	0.03	0.41–0.57	0.017	1.06	0.04	0.98–1.14
17β ol Δ^4 A 3 one	69	0.48	0.03	0.42–0.54	0.035			
3β 17β ol 5α A	30	0.48	0.03	0.41–0.55	0.035	0.99	0.03	0.93–1.05
3β 11β ol 5α A 17 one	9	0.44	0.03	0.37–0.53	0.105	0.90	0.07	0.73–1.07
3α 17β ol 5β A	12	0.44	0.03	0.38–0.50	0.105	0.88	0.04	0.80–0.96
3α 11β ol 5β A 17 one	9	0.43	0.04	0.34–0.52**	0.122	0.88	0.08	0.70–1.06
17β ol 5α A 3,7 one	8	0.40	0.02	0.37–0.43**	0.176	0.86	0.05	0.74–0.98
3α ol 5α A 7,17 one	8	0.40	0.02	0.35–0.45**	0.176	0.85	0.06	0.70–1.00
3α ol 5β A 11,17 one	9	0.38	0.04	0.29–0.47	0.213	0.78	0.07	0.61–0.95
3β ol 5α A 7,17 one	8	0.33	0.01	0.30–0.36	0.308	0.71	0.05	0.58–0.84

* System A (cyclohexane: 45, ethylacetate: 45, ethanol: 10).

** The employment of S.D. with three digits in the calculation of the F.L. results in slightly different F.L. in instances of identical R_F values, based on the same number of experiments and exhibiting the same S.D.

*** The number of experiments is n; S.D. indicates the standard deviation of a single estimation and is given in the tables to only two significant digits, whereas values with three digits were employed in the calculation of the fiducial limits (F.L.) — fiducial limits of error are those within which 95% of individual R_F values are expected to fall. These limits have been calculated as being on both sides of the mean R_F values are expected to fall. These limits have been calculated as being on both sides of the mean R_F-value to a distance of t × S.D., where t is the 95% probability for the corresponding degrees of freedom. The R_M value is defined as log $(1/R_F - 1)$.

TABLE III

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-SEVEN STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM C*

Steroid	*n***	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	12	0.73	0.02	0.68–0.78	—0.432	3.11	0.15	2.79–3.43
5α A 11 one	5	0.69	0.01	0.50–0.88	—0.338	3.19	0.11	2.88–3.50
5β A 11 one	5	0.69	0.02	0.64–0.74	—0.338	3.18	0.15	2.77–3.59
17β ol 5α A	6	0.54	0.02	0.48–0.60	—0.070	2.36	0.20	1.86–2.86
Δ^2 5α A 7,17 one	6	0.52	0.03	0.43–0.61	—0.035	2.19	0.08	1.99–2.39
5α A 3,17 one	8	0.46	0.02	0.42–0.50	0.070	1.87	0.13	1.54–2.20
5β A 3,17 one	12	0.43	0.02	0.38–0.48	0.122	1.83	0.09	1.63–2.03
Δ^1 5α A 3,17 one	6	0.38	0.03	0.30–0.46	0.213	1.61	0.09	1.38–1.84
3β ol 5β A 17 one	14	0.36	0.03	0.30–0.42	0.250	1.49	0.12	1.24–1.74
17β ol 5α A 3 one	18	0.35	0.03	0.29–0.41	0.269	1.48	0.09	1.28–1.68
3α ol 5α A 17 one	21	0.33	0.04	0.25–0.41	0.308	1.44	0.11	1.14–1.74
3β ol 5α A 17 one	24	0.30	0.03	0.23–0.37	0.368	1.23	0.11	1.00–1.46
17β ol 5β A 3 one	14	0.30	0.03	0.24–0.36	0.368	1.23	0.11	0.99–1.47
11β ol 5α A 3,17 one	19	0.27	0.04	0.19–0.35	0.432	1.13	0.08	0.96–1.30
3α 17β ol 5α A	5	0.25	0.02	0.21–0.29	0.477	1.16	0.14	0.76–1.56
3β 17β ol 5α A	12	0.25	0.02	0.20–0.30	0.477	1.05	0.07	0.90–1.20
3β 17β ol Δ^4 A	12	0.25	0.02	0.19–0.31	0.477	1.05	0.07	0.90–1.20
5β A 3,11,17 one	8	0.25	0.03	0.22–0.28	0.477	0.99	0.08	0.79–1.19
3α ol 5β A 17 one	21	0.24	0.03	0.18–0.30	0.501	0.92	0.09	0.74–1.10
17β ol Δ^4 A 3 one	54	0.23	0.02	0.19–0.27	0.525			
3α 17β ol 5β A	12	0.17	0.01	0.14–0.20	0.689	0.71	0.07	0.55–0.87
3β,11β ol 5α A 17 one	8	0.14	0.02	0.10–0.18	0.788	0.60	0.08	0.44–0.76
3α ol 5α A 7,17 one	6	0.12	0.02	0.08–0.16	0.865	0.50	0.08	0.30–0.70
17β ol 5α A 3,7 one	6	0.12	0.02	0.08–0.16	0.865	0.49	0.06	0.32–0.65
3α,11β ol 5β A 17 one	8	0.12	0.01	0.10–0.14	0.865	0.49	0.04	0.40–0.58
3α ol 5β A 11,17 one	8	0.09	0.01	0.06–0.12	1.005	0.38	0.05	0.27–0.49
3β ol 5α A 7,17 one	6	0.06	0.01	0.04–0.08	1.195	0.28	0.05	0.16–0.40

* System C: (ethyl acetate: 50, cyclohexane: 50).
** See footnote *** to Table II.

TABLE IV

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-FIVE STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM D*

Steroid	*n***	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	11	0.78	0.02	0.72–0.84	—0.550	1.35	0.04	1.30–1.40
Δ^2 5α A 7,17 one	7	0.77	0.03	0.71–0.82	—0.520	1.32	0.07	1.14–1.50
Δ^1 5α A 3,17 one	7	0.76	0.03	0.68–0.84	—0.501	1.30	0.08	1.11–1.49
5β A 3,17 one	12	0.74	0.04	0.66–0.82	—0.454	1.28	0.06	1.15–1.41
5α A 3,17 one	19	0.74	0.02	0.71–0.77	—0.454	1.27	0.07	1.13–1.41
5β A 3,11,17 one	12	0.69	0.05	0.58–0.80	—0.338	1.19	0.08	1.02–1.36
17β ol 5α A	6	0.64	0.02	0.60–0.68	—0.250	1.04	0.03	0.97–1.11
11β ol 5α A 3,17 one	12	0.63	0.04	0.54–0.72***	—0.231	1.08	0.06	0.95–1.21
3α ol 5α A 17 one	12	0.63	0.04	0.55–0.71***	—0.231	1.08	0.06	0.95–1.21
3β ol 5β A 17 one	12	0.62	0.03	0.56–0.68	—0.213	1.07	0.04	0.99–1.15
17β ol 5α A 3 one	12	0.61	0.04	0.52–0.70	—0.194	1.05	0.07	0.92–1.18
17β ol 5β A 3 one	12	0.61	0.02	0.56–0.66	—0.194	1.05	0.04	0.95–1.10
17β ol Δ^4 A 3 one	74	0.58	0.03	0.52–0.64	—0.140			
3α ol 5β A 17 one	12	0.58	0.03	0.51–0.65	—0.140	1.00	0.06	0.88–1.12

(continued on p. 397)

TABLE IV (*continued*)

Steroid	*n***	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
3β ol 5α A 17 one	12	0.58	0.03	0.52–0.64	—0.140	1.00	0.05	0.89–1.11
17β ol 5α A 3,7, one	7	0.56	0.05	0.44–0.68	—0.105	0.96	0.07	0.80–1.12
3α ol 5α A 7,17 one	7	0.53	0.05	0.40–0.66	—0.052	0.92	0.07	0.75–1.09
3α ol 5β A 11,17 one	12	0.52	0.03	0.45–0.59	—0.035	0.90	0.05	0.78–1.02
3β ol 5α A 7,17 one	7	0.50	0.05	0.37–0.63	0.000	0.86	0.07	0.70–1.02
3α 17β ol 5α A	12	0.50	0.03	0.43–0.57	0.000	0.86	0.04	0.78–0.94
3β,17β ol 5α A	23	0.47	0.04	0.39–0.55	0.052	0.81	0.07	0.67–0.95
3β,17β ol Δ^{4} A	12	0.47	0.04	0.37–0.57	0.052	0.81	0.05	0.70–0.92
3α,11β ol 5β A 17 one	12	0.44	0.03	0.38–0.50	0.105	0.76	0.04	0.68–0.84
3β,11β ol 5α A 17 one	12	0.43	0.03	0.37–0.49	0.122	0.74	0.04	0.65–0.83
3α,17β ol 5β A	12	0.40	0.04	0.31–0.49	0.176	0.69	0.06	0.55–0.83

* System D (chloroform: 90, ethanol: 10).
** See footnotes** and *** Table II.

TABLE V

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-FIVE STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM E*

Steroid	*n***	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	12	0.87	0.02	0.81–0.93	—0.826	1.29	0.03	1.24–1.34
Δ^{2} 5α A 7,17 one	8	0.81	0.02	0.76–0.86	—0.630	1.22	0.02	1.18–1.26
17β ol 5α A	8	0.78	0.04	0.69–0.87	—0.550	1.20	0.03	1.13–1.27
5β A 3,17 one	12	0.78	0.02	0.74–0.82	—0.550	1.15	0.03	1.09–1.21
5α A 3,17 one	9	0.77	0.04	0.58–0.86	—0.528	1.17	0.03	1.11–1.23
11β ol 5α A 3,17 one	9	0.75	0.03	0.67–0.83	—0.477	1.14	0.06	0.99–1.29
Δ^{1} 5α A 3,17 one	8	0.75	0.03	0.68–0.82	—0.477	1.18	0.04	1.04–1.22
3α ol 5α A 17 one	16	0.74	0.03	0.67–0.81	—0.454	1.14	0.04	1.06–1.22
17β ol 5α A 3 one	12	0.74	0.02	0.69–0.79	—0.454	1.10	0.03	1.03–1.17
3β ol 5β A 17 one	9	0.73	0.03	0.65–0.81	—0.432	1.11	0.02	1.08–1.14
17β ol 5β A 3 one	9	0.73	0.04	0.61–0.85	—0.432	1.11	0.04	1.02–1.20
3β ol 5α A 17 one	8	0.72	0.03	0.65–0.79	—0.410	1.08	0.02	1.04–1.12
3α ol 5β A 17 one	16	0.71	0.03	0.64–0.78	—0.389	1.09	0.03	1.05–1.13
5β A 3,11,17 one	9	0.70	0.03	0.63–0.77	—0.368	1.07	0.06	0.92–1.22
3α,17β ol 5α A	8	0.70	0.04	0.62–0.78	—0.368	1.05	0.03	0.98–1.12
3β,17β ol Δ^{4} A	12	0.70	0.03	0.64–0.76	—0.368	1.03	0.04	0.94–1.12
3β,11β ol 5α A 17 one	9	0.68	0.04	0.59–0.77	—0.327	1.04	0.07	0.87–1.21
3β,17β ol 5α A	23	0.68	0.04	0.60–0.76	—0.327	1.04	0.04	0.97–1.11
3α,11β ol 5β A 17 one	9	0.66	0.03	0.59–0.73	—0.288	1.00	0.05	0.88–1.12
17β ol Δ^{4} A 3 one	69	0.66	0.03	0.59–0.73	—0.288			
3α,17β ol 5β A	12	0.65	0.03	0.59–0.71	—0.269	0.97	0.04	0.89–1.05
3α ol 5β A 11,17 one	9	0.61	0.03	0.53–0.69	—0.194	0.93	0.05	0.81–1.05
17β ol 5α A 3,7 one	8	0.57	0.04	0.49–0.65	—0.122	0.86	0.04	0.77–0.95
3α ol 5α A 7,17 one	8	0.54	0.03	0.46–0.62	—0.070	0.82	0.04	0.63–0.91
3β ol 5α A 7,17 one	8	0.49	0.04	0.40–0.58	0.017	0.73	0.05	0.61–0.85

* System E (Ethyl acetate: 72, *n*-hexane: 13.5, ethanol: 4.5, acetic acid: 10).
** See footnote *** to Table II.

TABLE VI

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-FIVE STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM H

Steroid	n**	R_F	S.D.	F.L.	R_M	R_S	S.D.	F.L.
5α A 17 one	12	0.75	0.01	0.72–0.78	—0.477	1.34	0.05	1.23–1.45
Δ^2 5α A 7,17 one	8	0.70	0.04	0.62–0.78	—0.368	1.27	0.06	1.12–1.42
5β A 3,17 one	12	0.67	0.03	0.60–0.74	—0.308	1.21	0.04	1.11–1.31
Δ^1 5α A 3,17 one	8	0.66	0.02	0.61–0.71	—0.288	1.21	0.04	1.12–1.29
5β A 3,11,17 one	9	0.66	0.03	0.56–0.74***	—0.288	1.18	0.08	1.00–1.36
5α A 3,17 one	9	0.66	0.03	0.60–0.72***	—0.288	1.17	0.05	1.06–1.28
11β ol 5α A 3,17 one	9	0.64	0.04	0.56–0.72	—0.250	1.14	0.08	0.96–1.32
17β ol 5α A	6	0.62	0.02	0.56–0.68	—0.213	1.15	0.06	0.99–1.31
3β ol 5β A 17 one	17	0.59	0.03	0.54–0.64***	—0.158	1.03	0.04	0.95–1.11
17β ol 5β A 3 one	17	0.59	0.03	0.53–0.65***	—0.158	1.03	0.04	0.95–1.11
3α ol 5α A 17 one	22	0.58	0.02	0.53–0.65	—0.140	1.04	0.05	0.93–1.15
17β ol 5α A 3 one	20	0.57	0.01	0.54–0.60	—0.123	1.03	0.03	0.96–1.10
17β ol Δ^4 A 3 one	58	0.56	0.04	0.48–0.64	—0.105			
3α ol 5β A 17 one	22	0.56	0.04	0.48–0.64	—0.105	1.00	0.04	0.91–1.09
3β ol 5α A 17 one	17	0.55	0.02	0.50–0.60	—0.087	0.97	0.04	0.88–1.06
3α ol 5β A 11,17 one	9	0.55	0.02	0.49–0.61	—0.087	0.95	0.04	0.84–1.04
3α,11β ol 5β A 17 one	9	0.52	0.02	0.46–0.58	—0.035	0.89	0.05	0.78–1.00
3β,11β ol 5α A 17 one	9	0.51	0.03	0.44–0.58	—0.017	0.90	0.05	0.79–1.01
3β,17β ol Δ^4 A	12	0.50	0.02	0.45–0.55	0.000	0.89	0.03	0.82–0.96
17β ol 5α A 3,7, one	8	0.49	0.04	0.40–0.58	0.017	0.90	0.04	0.81–0.99
3β,17β ol 5α A	14	0.49	0.02	0.45–0.53	0.017	0.88	0.03	0.81–0.95
3α,17β ol 5α A	8	0.48	0.03	0.42–0.54	0.035	0.88	0.03	0.80–0.96
3α,17β ol 5β A	12	0.48	0.02	0.42–0.64	0.035	0.86	0.03	0.79–0.93
3α ol 5α A 7,17 one	8	0.47	0.04	0.38–0.56	0.052	0.85	0.06	0.71–0.99
3β ol 5α A 7,17 one	8	0.45	0.04	0.37–0.53	0.087	0.81	0.06	0.68–0.94

* System H (benzene: 40, ethanol: 10).
** and *** See footnotes ** and *** to Table II.

TABLE VII

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-SEVEN STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM K*

Steroid	n**	R_F	S.D.	F.L.	R_M	R_S	S.D.	F.L.
5α A 17 one	6	0.78	0.04	0.69–0.87	—0.550	2.16	0.19	1.68–2.64
5β A 11 one	6	0.73	0.02	0.68–0.78	—0.432	1.86	0.07	1.68–2.04
5α A 11 one	5	0.72	0.02	0.66–0.78	—0.432	1.85	0.08	1.62–2.08
5α A 3,17 one	7	0.63	0.04	0.53–0.73	—0.231	1.77	0.15	1.38–2.16
Δ^2 5α A 7,17 one	8	0.61	0.05	0.50–0.72	—0.194	1.70	0.18	1.24–2.16
5β A 3,17 one	18	0.60	0.03	0.53–0.67	—0.176	1.61	0.11	1.47–1.85
Δ^1 5α A 3,17 one	8	0.58	0.04	0.50–0.66	—0.140	1.61	0.13	1.30–1.92
17β ol 5α A	8	0.57	0.04	0.48–0.66	—0.122	1.27	0.08	1.06–1.48
5β A 3,11,17 one	18	0.50	0.04	0.41–0.59	0.000	1.32	0.09	1.13–1.51
3β ol 5β A 17 one	18	0.44	0.04	0.36–0.52***	0.105	1.16	0.03	1.10–1.22
11β ol 5α A 3,17 one	18	0.44	0.04	0.35–0.53***	0.105	1.15	0.03	1.09–1.21
3α ol 5α A 17 one	12	0.43	0.04	0.35–0.51	0.122	1.23	0.05	1.11–1.35
17β ol 5α A 3 one	6	0.42	0.03	0.35–0.49	0.140	1.18	0.07	1.00–1.36
17β ol 5β A 3 one	18	0.42	0.03	0.32–0.46	0.140	1.10	0.10	0.91–1.29
3β ol 5α A 17 one	18	0.39	0.03	0.32–0.46	0.194	1.04	0.06	0.91–1.17
17β ol Δ^4 A 3 one	49	0.39	0.04	0.31–0.49	0.194			

(continued on p. 399)

TABLE VII (*continued*)

Steroid	n**	R_F	S.D.	F.L.	R_M	R_S	S.D.	F.L.
3α ol 5β A 17 one	12	0.38	0.04	0.30–0.46	0.213	1.09	0.05	0.98–1.20
3α ol 5β A 11,17 one	18	0.33	0.04	0.24–0.42	0.308	0.88	0.07	0.73–1.03
3α,17β ol 5α A	18	0.32	0.04	0.24–0.40	0.327	0.84	0.06	0.66–1.02
3β,17β ol Δ⁴ A	15	0.30	0.06	0.18–0.42	0.368	0.75	0.09	0.56–0.94
17β ol 5α 3,7 one	8	0.29	0.04	0.19–0.39	0.389	0.80	0.09	0.58–1.02
3α,11β ol 5β A 17 one	18	0.29	0.04	0.20–0.38	0.389	0.77	0.07	0.62–0.92
3β,11β ol 5α A 17 one	18	0.28	0.04	0.19–0.37	0.410	0.74	0.08	0.57–0.91
3α ol 5α A 7,17 one	8	0.27	0.04	0.19–0.35	0.432	0.74	0.07	0.57–0.91
3β,17β ol 5α A	12	0.25	0.02	0.21–0.29	0.477	0.70	0.06	0.56–0.84
3β ol 5α A 7,17 one	8	0.23	0.03	0.15–0.31	0.525	0.65	0.07	0.48–0.82
3α,17β ol 5β A	6	0.23	0.01	0.20–0.26	0.525	0.65	0.02	0.60–0.70

* System K (benzene: 90, ethanol: 10).
** and *** See footnotes** and *** to Table II.

TABLE VIII

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-SEVEN STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM L*

Steroid	n**	R_F	S.D.	F.L.	R_M	R_S	S.D.	F.L.
5α A 17 one	12	0.76	0.03	0.69–0.83	—0.501	1.80	0.12	1.76–2.04
5α A 11 one	6	0.72	0.02	0.68–0.76	—0.410	1.65	0.12	1.31–1.99
5β A 11 one	6	0.71	0.03	0.63–0.79	—0.389	1.63	0.13	1.25–2.01
Δ² 5α A 7,17 one	7	0.70	0.03	0.63–0.77	—0.368	1.58	0.11	1.30–1.86
5α A 3,17 one	18	0.69	0.03	0.63–0.75	—0.338	1.64	0.15	1.33–1.95
5β A 3,17 one	12	0.67	0.04	0.57–0.77	—0.308	1.55	0.10	1.34–1.75
Δ¹ 5α A 3,17 one	8	0.67	0.02	0.65–0.69	—0.308	1.51	0.10	1.29–1.73
5β A 3,11,17 one	17	0.58	0.04	0.50–0.66	—0.140	1.36	0.12	1.10–1.66
17β ol 5α A	6	0.57	0.03	0.50–0.64	—0.122	1.30	0.04	1.19–1.41
3β ol 5β A 17 one	18	0.50	0.03	0.43–0.57	—0.000	1.20	0.12	0.96–1.44
3α ol 5α A 17 one	15	0.50	0.05	0.39–0.61	0.000	1.18	0.10	0.97–1.49
17β ol 5α A 3 one	12	0.49	0.03	0.42–0.56	0.017	1.19	0.07	1.04–1.34
11β ol 5α A 3,17 one	18	0.46	0.04	0.39–0.51	0.070	1.10	0.10	0.90–1.30
17β ol 5β A 3 one	17	0.46	0.04	0.38–0.54	0.070	1.10	0.11	0.87–1.33
3β ol 5α A 17 one	18	0.44	0.04	0.36–0.52	0.105	1.04	0.06	0.92–1.16
3α ol 5β A 17 one	15	0.43	0.05	0.31–0.55	0.122	1.02	0.10	0.71–1.23
17β ol Δ⁴ A 3 one	64	0.43	0.08	0.27–0.59	0.122			
17β ol 5α A 3,7 one	7	0.36	0.05	0.23–0.49	0.250	0.81	0.10	0.56–1.06
3α,17β ol 5α A	18	0.32	0.05	0.22–0.42	0.327	0.77	0.11	0.55–0.99
3β,17β ol Δ⁴ A	21	0.32	0.04	0.25–0.39	0.327	0.75	0.06	0.59–0.91
3α ol 5α A 7,17 one	7	0.32	0.03	0.24–0.40	0.327	0.70	0.05	0.57–0.83
3α ol 5β A 11,17 one	18	0.31	0.04	0.24–0.38	0.348	0.71	0.06	0.59–0.83
3β 17β ol 5α A	12	0.29	0.03	0.22–0.36	0.389	0.70	0.06	0.56–0.84
3β ol 5α A 7,17 one	7	0.27	0.05	0.16–0.38	0.432	0.61	0.09	0.39–0.83
3α,17β ol 5β A	12	0.23	0.02	0.18–0.28	0.525	0.56	0.04	0.47–0.64
3α,11β ol 5β A 17 one	18	0.23	0.03	0.16–0.30	0.525	0.54	0.11	0.31–0.77
3β,11β ol 5α A 17 one	18	0.22	0.03	0.16–0.28	0.550	0.53	0.07	0.38–0.68

* System L (chloroform: 19, ethanol: 1).
** See footnote *** to Table II.

TABLE IX

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-FIVE STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM M*

Steroid	*n***	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	8	0.80	0.03	0.73–0.87	—0.602	1.56	0.06	1.43–1.69
Δ² 5α A 7,17 one	8	0.74	0.03	0.64–0.81	—0.454	1.43	0.05	1.30–1.56
17β ol 5α A	6	0.73	0.01	0.70–0.76	—0.432	1.44	0.09	1.21–1.67
5α A 3,17 one	8	0.72	0.02	0.68–0.76	—0.410	1.35	0.08	1.21–1.49
5β A 3,17 one	8	0.69	0.02	0.64–0.73	—0.338	1.33	0.07	1.13–1.49
Δ¹ 5α A 3,17 one	8	0.67	0.04	0.58–0.76	—0.308	1.30	0.04	1.20–1.40
3β ol 5β A 17 one	8	0.66	0.02	0.61–0.71	—0.288	1.26	0.05	1.15–1.37
17β ol 5β A 3 one	8	0.63	0.02	0.58–0.68	—0.231	1.19	0.05	1.08–1.30
17β ol 5α A 3 one	8	0.62	0.02	0.58–0.66	—0.213	1.20	0.05	1.09–1.31
3α ol 5α A 17 one	8	0.62	0.03	0.55–0.69	—0.213	1.20	0.06	1.06–1.34
3β ol 5α A 17 one	8	0.61	0.04	0.51–0.71	—0.194	1.17	0.04	1.07–1.27
11β ol 5α A 3,17 one	8	0.59	0.02	0.55–0.63	—0.158	1.15	0.04	1.06–1.24
3β,17β ol Δ⁴ A	8	0.58	0.01	0.55–0.61	—0.140	1.12	0.05	1.00–1.24
3α ol 5β A 17 one	8	0.57	0.02	0.53–0.61	—0.122	1.11	0.05	0.98–1.20
3α,17β ol 5α A	8	0.56	0.02	0.52–0.60	—0.105	1.09	0.05	0.98–1.18
5β A 3,11,17 one	8	0.56	0.01	0.54–0.58	—0.105	1.08	0.04	0.92–1.18
3β,17β ol 5α A	8	0.54	0.02	0.49–0.59	—0.070	1.05	0.06	0.92–1.18
17β ol Δ⁴ 3 one	34	0.52	0.02	0.47–0.57	—0.035			
3β,11β ol 5α A 17 one	8	0.51	0.01	0.48–0.53	—0.017	0.98	0.05	0.87–1.09
3α,17β ol 5β A	8	0.50	0.02	0.45–0.55	0.000	0.96	0.04	0.86–1.06
3α,11β ol 5β A 17 one	7	0.48	0.01	0.45–0.51	0.035	0.92	0.05	0.80–1.04
3α ol 5β A 11,17 one	7	0.43	0.02	0.39–0.47	0.122	0.82	0.05	0.70–0.94
17β ol 5α A 3,7 one	8	0.42	0.02	0.37–0.47	0.140	0.82	0.04	0.74–0.90
3α ol 5α A 7,17 one	8	0.40	0.02	0.35–0.46	0.176	0.77	0.03	0.71–0.83
3β ol 5α A 7,17 one	8	0.34	0.02	0.29–0.39	0.288	0.65	0.03	0.58–0.72

* System M (ethyl acetate: 75, *n*-hexane: 20, acetic acid: 5).
** See footnote *** to Table II.

TABLE X

R_F, R_S (S = TESTOSTERONE) AND FUNCTION R_M OF TWENTY-SEVEN STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM N*

Steroid	*n***	R_S	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
5α A 17 one	13	0.66	0.04	0.58–0.74	—0.288	2.89	0.24	2.37–3.41
5β A 11 one	5	0.66	0.03	0.58–0.74	—0.288	2.87	0.31	2.00–3.74
5α A 11 one	5	0.66	0.03	0.57–0.75	—0.288	2.86	0.32	1.97–3.75
Δ² 5α A 7,17 one	8	0.55	0.02	0.46–0.64	—0.087	2.41	0.21	1.91–2.91
5α A 3,17 one	8	0.53	0.06	0.44–0.70	—0.052	2.06	0.15	1.71–2.41
5β A 3,17 one	8	0.49	0.04	0.40–0.58	0.017	2.11	0.22	1.59–2.63
17β ol 5α A	10	0.46	0.06	0.31–0.61	0.070	1.75	0.15	1.42–2.08
Δ¹ 5α A 3,17 one	8	0.45	0.02	0.41–0.49	0.087	1.97	0.15	1.61–2.33
5β A 3,11,17 one	8	0.36	0.04	0.27–0.45	0.250	1.60	0.18	1.18–2.02
3β ol 5β A 17 one	9	0.33	0.04	0.23–0.43	0.308	1.27	0.10	1.05–1.49
17β ol 5α A 3 one	10	0.31	0.02	0.26–0.36	0.348	1.31	0.12	1.04–1.58
3α ol 5α A 17 one	21	0.29	0.03	0.22–0.36	0.389	1.29	0.16	0.96–1.62
17β ol 5β A 3 one	8	0.28	0.02	0.23–0.33	0.410	1.11	0.09	0.90–1.32
3β ol 5α A 17 one	10	0.27	0.02	0.22–0.32	0.432	1.15	0.10	0.92–1.38
11β ol 5α A 3,17 one	8	0.27	0.03	0.19–0.35	0.432	1.22	0.05	1.10–1.36
17β ol Δ⁴ A 3 one	47	0.24	0.03	0.19–0.29	0.501			

(continued on p. 401)

TABLE X (*continued*)

Steroid	n**	R_F	*S.D.*	*F.L.*	R_M	R_S	*S.D.*	*F.L.*
3α ol 5β A 17 one	11	0.23	0.03	0.17–0.29	0.525	1.07	0.11	0.85–1.29
3α,17β ol 5α A	18	0.18	0.02	0.14–0.22	0.659	0.80	0.07	0.65–0.95
17β ol 5α A 3,7 one	8	0.18	0.02	0.13–0.23	0.659	0.79	0.07	0.62–0.96
3α ol 5β A 11,17 one	9	0.18	0.03	0.11–0.25	0.659	0.71	0.07	0.55–0.87
3β,17β ol Δ^4 A	14	0.17	0.02	0.13–0.21	0.689	0.74	0.07	0.60–0.88
3β,17β ol 5α A	16	0.16	0.02	0.12–0.20	0.720	0.71	0.02	0.67–0.75
3α ol 5α A 7,17 one	8	0.16	0.02	0.12–0.20	0.720	0.70	0.04	0.59–0.81
3α,11β ol 5β A 17 one	8	0.13	0.02	0.09–0.17	0.826	0.53	0.05	0.41–0.65
3β ol 5α A 7,17 one	8	0.13	0.02	0.09–0.17	0.826	0.57	0.05	0.45–0.69
3α,17β ol 5β A	8	0.13	0.02	0.09–0.17	0.826	0.55	0.05	0.43–0.67
3β,11β ol 5α A 17 one	9	0.13	0.02	0.08–0.18	0.826	0.53	0.06	0.39–0.67

* System N (benzene: 19, ethanol: 1).
** See footnote *** to Table II.

The situation is the same if the ring has only one ketonic group: 5α- and 5β-dihydro-testosterone may be separated using system C, while in systems L and N only a partial separation is obtained.

The introduction of an unsaturated bond altered the mobility of very few steroids. R_F values of Δ^1-dehydro-androstanedione, androstanedione and etiocholanedione are very similar; their complete separation is, however, accomplished in systems C and N, and is especially good in system O. The separation of androst-4-ene-3β,17β-diol from the different epimeric forms of the androstanediols is equally difficult. It is possible to separate it from the isomers with a 3-equatorial hydroxyl group (3α,5β and 3β,5α) in system K, but not from 5α-androstane-3α,17β-diol; this separation is partially achieved in system D (R_F values: 0.47 and 0.50 respectively).

The situation is changed when a conjugated double bond is introduced: testosterone and 5α- or 5β-dihydro-testosterone may be satisfactorily separated in nearly all

TABLE XI

R_F, AND R_S (S = ANDROSTANEDIONE) OF ELEVEN STEROIDS ON THE C_{19} AND ONE ON THE C_{18} (NOR-SERIES) IN SOLVENT SYSTEM O*, USING THIN-LAYER CHROMATOGRAPHY (COMPLETE SATURATION) ON SILICA GEL G (MERCK A.G., BATCH NO. 62631)

Steroid	n**	R_F	*S.D.*	R_S	*S.D.*
5β A 11 one	5	0.64	0.02	3.81	0.25
5α A 11 one	5	0.64	0.02	3.78	0.25
5α A 17 one	9	0.55	0.02	3.20	0.22
17β ol 5α A	9	0.31	0.02	1.82	0.07
Δ^2 5α A 7,17 one	9	0.27	0.03	1.56	0.09
5α A 3,17 one	9	0.17	0.01	1.00	
5β A 3,17 one	9	0.14	0.01	0.82	0.02
Δ^1 5α A 3,17 one	9	0.13	0.01	0.75	0.02
Δ^4 A 3,17 one	6	0.08	0.008	0.44	0.04
19-nor Δ^4 A 3,17 one	6	0.06	0.006	0.32	0.02
5β A 3,11,17 one	8	0.04	0.006	0.23	0.03
17β ol Δ^4 A 3 one	9	0.04	0.006	0.24	0.04

* System O (n-hexane: 75, ethyl acetate: 25).
** See footnote*** to Table II.

the systems, especially in systems E, A, C, N, and M (Tables II, III, V, IX and X). Another example of this is the separation of androstenedione and androstanedione or etiocholanedione in system O (Table XI).

In the case of isomers containing a 3-hydroxyl group, the separation depends upon the spatial configuration, an equatorial group being easily separated from an axial one, while the separation of two axial or equatorial isomers is very difficult.

In nearly all of the proposed systems it is possible to separate androsterone and 5β-epiandrosterone from etiocholanolone and epiandrosterone, complete separation being possible in systems C, D, H, K and L. The separation of both pairs from each other can be achieved in solvent systems C, N and M (Tables III, IX and X).

The same observation holds true for 11β-hydroxy-etiocholanolone and 11β-hydroxy-epiandrosterone, both containing a 3-hydroxyl group with an equatorial configuration. Their separation is only partially achieved in solvent system M, (R_F values: 0.51 and 0.48 respectively). On the other hand, 7-oxo-androsterone (axial) and 7-oxo-epiandrosterone (equatorial) are separable in all the systems studied, with the exception of system O, in which they remain at the origin.

Of the androstanediols studied, the complete separation of the axial isomer 5α-androstane-3α,17β-diol, from 5α-androstane-3β,17β-diol and 5β-androstane-3α,17β-diol, both equatorials, is possible in systems K and D (Tables IV and VII). The separation of the latter two is also achieved in systems D, L, N and M (Tables IV, VIII, IX and X).

The calculated ΔR_{Mg} values for some groups, *viz.* 3α-(axial), 3β-hydroxy-(equatorial), 3-oxo-, 7-oxo-, 11β-hydroxy- and 11-oxo-, are summarised in Table XII.

It may be noted that the sequence of polarity for 3-oxygenate derivatives is 3-oxo- < 3α-hydroxyl (axial) < 3β-hydroxyl (equatorial) in the majority of systems while in a few a reversal of polarities occurs. An 11β-hydroxyl group with rings A/B in the *cis*-configuration is less polar than the isomer with rings A/B in the *trans*-configuration. An 11-oxo group is more polar than an 11-hydroxy group in systems containing ethyl acetate and less polar in systems where benzene or chloroform is the principal component. This fact is of great value in the selection of systems for two-dimensional chromatography, because systems may be selected, which produce this reversal of polarity in the groups to be separated. This reversal can also be useful in the separation and identification of many substances, *e.g.* 11-oxo-etiocholanolone and 11β-hydroxy-etiocholanolone.

The ΔR_{Mr} obtained for the conversion of the group 11-oxo- to 11β-hydroxy- were negative in systems containing ethyl acetate and cyclo- or *n*-hexane: —0.10 (system E), —0.10 (system A), and —0.14 (system C), while positive values were obtained in systems containing benzene or chloroform: +0.05, +0.08, +0.14, +0.17 and +0.17 respectively for systems H, K, D, N and L.

The calculated ΔR_{Ms} values of 26 steroids for the pairs of systems E and C (I), A and L (II) and L and K (III) have been collected in Table XIII.

It can be seen that the introduction of various groups into the steroid molecule causes changes in polarity (ΔR_{Ms} values) with respect to the three solvent systems described. One such example is androsterone, the ΔR_{Ms} being greater for the change from system E to D (I) (+0.22) than for the changes (II) and (III) (+ 0.09 and +0.12 respectively). The reduction of the 17-oxo group results in ΔR_{Ms} values which are greater than those above, but almost identical for both changes (I) and (II),

TABLE XII

ΔR_{Mg} VALUES OF SOME HYDROXYL AND KETONIC GROUPS IN THE ANDROSTANE SERIES, CALCULATED FOR THE EIGHT PRINCIPAL SYSTEMS. $\Delta R_{Mg} = R_{M1} - R_{M2}$, WHERE THE R_{M1} IS THE R_M OF THE SUBSTANCE WITH THE GROUP UNDER CONSIDERATION AND R_{M2} IS THAT OF THE SUBSTANCE WITHOUT THESE GROUPS. *a* AND *e* SIGNIFY AXIAL AND EQUATORIAL

Group	*Compound in which radical is substituted*	*Remarks*	*System*								
			A	*C*	*D*	*E*	*H*	*K*	*L*	*M*	*N*
3-oxo	17β ol 5α A	Ring A/B *trans*	0.13	0.34	0.06	0.10	0.09	0.26	0.14	0.22	0.34
3β-OH	17β ol 5α A	Ring A/B *trans* 3β (*e*)	0.28	0.55	0.30	0.22	0.23	0.60	0.51	0.36	0.65
3α-OH	17β ol 5α A	Ring A/B *trans* 3α (*a*)	0.25	0.55	0.25	0.18	0.25	0.45	0.45	0.33	0.59
11β-OH	5α A 3,17 one	Ring A/B *trans* 3-oxo	0.11	0.36	0.22	0.05	0.04	0.28	0.41	0.25	0.48
	3β ol 5α A 17 one	Ring A/B *trans* 3β (*e*)	0.14	0.42*	0.26	0.08	0.07	0.22	0.45	0.18	0.39
	3α ol 5β A 17 one	Ring A/B *cis* 3α (*e*)	0.10	0.36*	0.24	0.10	0.07	0.18	0.40	0.16	0.30
11-oxo	3α ol 5β A 17 one	Ring A/B *cis* 3α (*e*)	0.20	0.50*	0.10	0.20	0.02	0.10	0.23	0.24	0.13
	5β A 3,17 one	Ring A/B *cis* 3-oxo	0.22	0.36	0.12	0.18**	0.02	0.18	0.17	0.23	0.23
7-oxo	17β ol 5α A 3 one	Ring A/B *trans* 3-oxo	0.30	0.60*	0.09	0.33	0.14	0.25	0.23	0.35	0.31
	3α ol 5α A 17 one	Ring A/B *trans* 3α (*a*)	0.20	0.56*	0.18	0.38	0.19	0.31	0.33	0.39	0.33
	3β ol 5α A 17 one	Ring A/B *trans* 3β (*e*)	0.34	0.83*	0.14	0.39	0.17	0.33	0.32	0.48	0.39

* Value calculated with an R_F under 0.15.
** Value calculated with one or both R_F values over 0.75.

TABLE XIII

ΔR_{Ms} VALUES FOR CHANGES FROM SYSTEMS E TO D (I), A TO L (II) AND L TO K (III) FOR TWENTY-SIX C_{19}-STEROIDS

Steroid	ΔR_{Ms}: *(I)*	*(II)*	*(III)*
Steroids without hydroxy-groups			
5α A 17 one	+0.276	−0.024	−0.049
5α A 3,17 one	+0.074	−0.107	+0.107
5β A 3,17 one	+0.096	−0.058	+0.132
Δ^1 5α A 3,17 one	−0.024	−0.114	+0.168
Δ^4 A 3,17 one	−0.086	−0.217	+0.199
Δ^2 5α A 7,17 one	+0.110	−0.060	+0.174
5β A 3,11,17 one	+0.030	−0.105	+0.140
Monohydroxy-C_{19}-steroids with a 3- or 17-oxo-group			
17β ol 5α A 3 one	+0.260	+0.140	+0.122
17β ol 5β A 3 one	+0.274	+0.157	+0.070
17β ol Δ^4 A 3 one	+0.148	+0.087	+0.072
3α ol 5α A 17 one	+0.223	+0.087	+0.122
3β ol 5α A 17 one	+0.270	+0.140	+0.089
3α ol 5β A 17 one	+0.249	+0.104	+0.091
3β ol 5β A 17 one	+0.219	+0.122	+0.105
17-Hydroxy-steroids without oxo-groups			
17β ol 5α A	+0.300	+0.128	0.000
3α 17β ol 5α A	+0.368	+0.327	0.000
3β 17β ol 5α A	+0.379	+0.354	+0.088
3β 17β ol Δ^4 A	+0.420	+0.327	+0.041
3α 17β ol 5β A	+0.445	+0.420	0.000
C_{19}-steroids with an 11β- hydroxy-group			
11β ol 5α A 3,17 one	+0.246	+0.192	+0.035
3β11β ol 5α A 17 one	+0.449	+0.445	−0.140
3α11β ol 5β A 17 one	+0.393	+0.403	−0.136
3- or 17-hydroxy-C_{19}-steroids with a 7- or 11-oxo-group			
3α ol 5β A 11,17 one	+0.159	+0.135	−0.040
17β ol 5α A 3,7 one	+0.017	+0.074	+0.139
3α ol 5α A 7,17 one	+0.018	+0.151	+0.105
3β ol 5α A 7,17 one	−0.018	+0.124	+0.093

(+0.37 and +0.33), while no change at all is observed for the pair of solvent systems (III).

When a 7-oxo group is introduced into the molecule, a comparison of mobilities in the three pairs of systems considered above shows ΔR_{Ms} values characteristic for this group, *i.e.* a decrease in the change (I), +0.018, and almost no alteration for the changes (II) and (III), when these are compared with those given by androsterone, +0.15 and +0.11, respectively.

The ΔR_{Ms} values for the change from system E to A show that the introduction of a double bond increases these values, while for the introduction of a conjugated double bond the opposite occurs. One may also note that the introduction of an hydroxyl group (3α- 3β- 11β-), or a reduction of the 17-oxo group increases the ΔR_{Ms} in change (I), while the introduction of the groups 7- or 11-oxo- decreases the values.

Formation of acetate derivatives

0.5 ml of pyridine and 0.5 ml of acetic anhydride is added to 1–20 µg of steroid in a ground-glass tube. The tube is sealed under an atmosphere of N_2 and allowed to stand at room temperature overnight. Under these conditions a 3α-, 3β- and 17β-, but not 11β-hydroxyl groups, are completely acetylated.

The R_F, R_S (S = testosterone) and R_M values for solvent system C of the seventeen acetates obtained are shown in Table XIV. A partial separation is possible of the acetates of 5α- and 5β-dihydro-testosterone from those of the isomeric forms of 3-hydroxy-androstan-17-one. However, no separation is obtained among the isomers of the individual groups. Similarly, the almost identical R_F values of the various acetates of androstanediol isomers do not allow satisfactory separation.

TABLE XIV

R_F, R_S (S = TESTOSTERONE) AND R_M VALUES FOR SEVENTEEN ACETATES OF STEROIDS OF THE ANDROSTANE SERIES IN SOLVENT SYSTEM C*, USING THIN-LAYER CHROMATOGRAPHY AT COMPLETE SATURATION, ON SILICA GEL G (MERCK A.G., BATCH NO. 62631)

Acetate of	*n***	R_F	*S.D.*	R_M	R_S	*S.D.*
17β ol 5α A	11	0.67	0.03	—0.308	3.19	0.22
3α 17β ol 5β A	11	0.65	0.03	—0.269	3.05	0.18
3β 17β ol Δ⁴ A	11	0.63	0.03	—0.231	2.94	0.17
3α 17β ol 5α A	11	0.62	0.03	—0.213	2.95	0.22
3β 17β ol 5α A	11	0.60	0.04	—0.176	2.84	0.20
3α ol 5β A 17 one	11	0.56	0.03	—0.105	2.65	0.16
3β ol 5α A 17 one	11	0.55	0.03	—0.087	2.63	0.21
3α ol 5α A 17 one	11	0.55	0.03	—0.087	2.60	0.18
3β ol 5β A 17 one	11	0.55	0.02	—0.087	2.60	0.16
17β ol 5α A 3 one	11	0.52	0.03	—0.035	2.52	0.21
17β ol 5β A 3 one	11	0.52	0.03	—0.035	2.47	0.19
3α 11β ol 5β A 17 one	11	0.45	0.03	0.087	2.12	0.10
3α ol 5β A 11,17 one	11	0.44	0.02	0.105	2.10	0.12
3β 11β ol 5α A 17 one	11	0.41	0.03	0.158	2.00	0.16
3β ol 5α A 7,17 one	11	0.38	0.04	0.213	1.75	0.18
3α ol 5α A 7,17 one	11	0.35	0.02	0.269	1.65	0.12
17β ol 5α A 3,7 one	11	0.30	0.02	0.368	1.41	0.08

* System C (ethyl acetate: 50, cyclohexane: 50).
** See footnote *** to Table II.

Other solvents[7] such as benzene, chloroform, 1,2-dichloromethane and tetrachloromethane, or mixtures of solvents[1] (benzene–ethyl acetate 5:1 and 3:1) have been employed for thin-layer chromatography of weakly polar steroids or their acetates. They did not satisfactorily separate the acetates considered in this paper.

DISCUSSION

Partition paper chromatography has been widely applied to the separation of steroids of the androstane series using formamide[2,21], propylene glycol[2,16,22], triethylene glycol[31], or ethylene glycol[30] as stationary phases. These systems permit separation of the axial and equatorial isomeric forms of the 3-hydroxy derivatives, but two-axial or two-equatorial isomers are not satisfactorily separated.

The extensive experiments of KNUPPEN[15] to achieve separation of oestrogens using these systems have shown that the R_F values and resolution obtained for many of the compounds were greatly dependent both upon the humidity of the atmosphere during impregnation, and upon the temperature during the development of the chromatogram. The impregnated paper usually interferes with several of the colour reactions employed. However, the sensitivity of the Zimmermann reaction appears to be increased when triethylene glycol impregnated paper is used[31].

When thin-layer chromatography is applied to the free steroids of the androstane series it results in a total or partial separation of several isomers with reproducibility of the R_F and consequently of the ΔR_M functions.

A quantitative elution (95 %) of added steroid, measured by the Zimmermann reaction and the possibility of the application of several colour reactions *in situ* are other important advantages.

With regard to the separation of acetates, however, the results obtained by PASQUALINI AND JAYLE[25] using paper adsorption chromatography are more satisfactory than those obtained with the systems developed for thin-layer chromatography.

In paper chromatography, the conditions necessary to obtain reliable values of R_F which will permit calculation of the comparable ΔR_M functions have been studied intensively by GREEN AND MARCINKIEWICZ[13]. There has been similar study for thin-layer chromatography (an adsorption chromatography of ascending type). The ΔR_M values calculated here with an R_F higher than 0.75 and lower than 0.15 are considered to be approximate.

The R_M values given in each Table permit the calculation of the different ΔR_{Mg}, ΔR_{Ms} and ΔR_{Mr} for several steroids and systems. The relation between these functions and the application of chromatography to the structural analyses of steroids[5, 14, 20] has been extensively treated by others.

While this paper was in preparation, an article[8] appeared considering the application of thin-layer chromatography to androstane steroids using silica gel C as an adsorbent under unsaturated conditions.

Whenever extremely volatile solvents are employed, the irregular migration of a substance, dependent on its position at the origin (the so-called "Randeffekt" or edge-phenomenon), may be observed during thin-layer chromatography under unsaturated conditions. R_F values are dependent upon the distance from the origin to the solvent front and are influenced by the kind and the size of tank employed[23]. The compression of less polar steroids depends upon the irregularity of saturation along the chromatoplate even in such instances where no "Randeffekt" is observed.

Under these conditions, the resulting variations in R_F values do not allow the accurate calculation of the function R_M.

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SUMMARY

The behaviour of twenty-nine steroids of the androstane series was studied by thin-layer chromatography on Silica gel G under saturated conditions. Besides the R_F and R_S values in ten systems, the chromatographic behaviour of the steroids in relationship to their structure was also studied. In a few cases, examples using ΔR_{Mg}, ΔR_{Ms} and ΔR_{Mr} are given.

Chromatography of acetate derivatives, oxidation *in situ* of hydroxy groups and a number of colour reactions employed for the detection of spots are also described.

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