CHROM. 3797

APPLICATION OF ASCENDING THIN-LAYER CHROMATOGRAPHY TO Δ^5-3-HYDROXYSTEROIDS OF THE PREGNANE SERIES

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(Received September 26th, 1968)

SUMMARY

The chromatographic behaviour of thirty-one Δ^5-3-hydroxy-C_{21}-steroids on analytical Silica Gel G-layers was investigated on eight solvent systems.

For the separation of closely related alcoholic trihydroxysteroids, one-dimensional ascending thin-layer chromatography on boric acid-impregnated silica gel layers has been used. Pregnenolone and 16-dehydropregnenolone have been resolved by elatography after formation of isonicotinic acid hydrazones. The separation of the epimeric Δ^5-pregnene-3β,20-diols was only achieved after treatment with 20β-hydroxysteroid-oxidoreductase.

INTRODUCTION

Ascending thin-layer chromatography on silica gel layers has been widely used for the analyses of mixtures of steroids and sterols[1,2]. A systematic method for analyses of steroids on thin-layer chromatography has been developed by the author[3] for steroids of the oestrane[4], androstane[5–7] and pregnane series[8–10].

In the present communication the extension of these investigations to the steroids of the pregnane series with a Δ^5-3-hydroxy-structure is reported. Besides the use of the solvent systems and colour reactions previously developed, some new techniques have been proposed for the separation of some "critical pairs" of steroids of this series.

MATERIALS AND METHODS

All the reagents used throughout this investigation were of analytical grade and their sources have been indicated in previous communications[4–10]. The sources and systematic names of the Δ^5-3-hydroxy-C_{21}-steroids used in this work are listed in Table I.

Ascending one-dimensional thin-layer chromatography on Silica Gel G (Merck-A.G., Darmstadt, Germany) was used according to the experimental conditions previously described[6,9] in rectangular tanks (21 × 21 × 9 cm, Desaga, Heidelberg,

TABLE I

SYSTEMATIC NAMES, ABBREVIATIONS, SOURCES AND COLOURS DEVELOPED WITH THE ANISALDEHYDE–SULPHURIC ACID REAGENT FROM THE THIRTY-ONE STEROIDS CONSIDERED IN THIS INVESTIGATION

Systematic names	*Abbreviations*	*Sources*	*Colour reaction*
3β-Hydroxy-pregn-5-en-20-one	3β-ol-P^5-20-one	h	green
3β-Hydroxy-pregna-5,16-dien-20-one	3β-ol-$P^{5,16}$-20-one	c	grey-violet
3β,7α-Dihydroxy-pregn-5-en-20-one	3β,7α-ol-P^5-20-one	k	olive-green
3β,7α-Dihydroxy-pregn-5-en-20-one,3β,7α-diacetate	3β,7α-ol-P^5-20-one,Ac.	h,k	olive-green
3β,15α-Dihydroxy-pregn-5-en-20-one	3β,15α-ol-P^5-20-one	j	bluish green to violet
3β,16α-Dihydroxy-pregn-5-en-20-one	3β,16α-ol-P^5-20-one	m	grey-violet to blue
3β,17α-Dihydroxy-pregn-5-en-20-one	3β,17α-ol-P^5-20-one	c,g	olive to olive-brown
3β-21-Dihydroxy-pregn-5-en-20-one	3β,21-ol-P^5-20-one	h	grey-violet to blue
3β,17α,20α-Trihydroxy-pregn-5-en-11-one	3β,17α,20α-ol-P^5-11-one	d	olive
3β,11β,17α-Trihydroxy-pregn-5-en-20-one	3β,11β,17α-ol-P^5-20-one	d	olive-grey
3β,16α,17α-Trihydroxy-pregn-5-en-20-one	3α,16α,17α-ol-P^5-20-one	a	bluish violet to violet-brown
3β,16α,17α-Trihydroxy-pregn-5-en-20-one,16α-acetate	3β,16α,17α-ol-P^5-20-one,16α-Ac.	a	bluish violet to violet brown
3β,17α,21-Trihydroxy-pregn-5-en-20-one	3β,17α,21-ol-P^5-20-one	l	blue-violet to grey-blue
3β,17α,21-Trihydroxy-pregn-5-en-20-one,21-acetate	3β,17α,21-ol-P^5-20-one,21-Ac.	m	blue-violet to grey-blue
3β-Hydroxy-pregn-5-ene-15,20-dione	3β-ol-P^5-15,20-one	j	pale violet to lilac
3β,11α-Dihydroxy-pregn-5-ene-7,20-dione	3β,11α-ol-P^5-7,20-one	i	yellow
Pregn-5-en-3β-ol	3β-ol-P^5	b	grey-blue
Pregn-5-ene-3β,20α-diol	3β,20α-ol-P^5	h	blue
Pregn-5-ene-3β,20β-diol	3β,20β-ol-P^5	h	blue
Pregn-5-ene-3β,15α,20α-triol	3β,15α,20α-ol-P^5	n	reddish lilac to violet-blue
Pregn-5-ene-3β,15α,20β-triol	3β,15α,20β-ol-P^5	j	reddish lilac to violet-blue
Pregn-5-ene-3α,16α,20α-triol	3α,16α,20α-ol-P^5	e	pale ultramarine
Pregn-5-ene-3α,16α,20β-triol	3α,16α,20β-ol-P^5	e	pale ultramarine
Pregn-5-ene-3β,16α,20α-triol	3β,16α,20α-ol-P^5	e,q	ultramarine
Pregn-5-ene-3β,16α,20β-triol	3β,16α,20β-ol-P^5	e,q	ultramarine
Pregn-5-ene-3β,17α,20α-triol	3β,17α,20α-ol-P^5	o	blue to sea-green
Pregn-5-ene-3β,17α,20β-triol	3β,17α,20β-ol-P^5	o	blue to sea-green
Pregn-5-ene-3β,18,20β-triol	3β,18,20β-ol-P^5	f	olive-green
Pregn-5-ene-3β,20α,21-triol	3β,20α,21-ol-P^5	p	blue
Pregn-5-ene-3β,20β,21-triol	3β,20β,21-ol-P^5	p	blue
Pregn-5-ene-3β,11β,17α,20α-tetrol	3β,11β,17α,20α-ol-P^5	d	blue-violet

Sources: (a) Dr. A. A. AKHREM, Moscow, U.S.S.R. (b) Dr. S. BERNSTEIN, Pearl River, U.S.A. (c) Dr. O. A. DE BRUIN, Weesp, The Netherlands. (d) Dr. M. FINKELSTEIN, Jerusalem, Israel, by courtesy of Dr. R. I. Cox, Australia. (e) Dr. D. FUKUSHIMA, New York, U.S.A. (f) Dr. J. JOSKA, Prague, Czechoslovakia. (g) Prof. K. JUNKMANN, Berlin, Germany. (h) Prof. W. KLYNE, London, England. (i) Dr. L. KOGAN, Moscow, U.S.S.R. (j) Dr. G. SNATZKE, Bonn, Germany. (k) Dr. L. STARKA, Prague, Czechoslovakia. (l) U.S.P. Steroid Reference Substance. (m) Steraloids Inc., Flushing 52, New York, U.S.A. (n) Obtained by the reduction of 3β,15α-dihydroxy-pregn-5-en-20-one with lithium aluminum hydride in tetrahydrofuran, followed by separation of both isomers using thin-layer chromatography (system I). (o) Obtained by the reduction of 3β,17α-dihydroxy-pregn-5-en-20-one, as described in (m). (p) Obtained by the reduction of 3β,21-dihydroxy-pregn-5-en-20-one, as described in (n); the isomers were chromatographed without previous separation. (q) Obtained by the reduction of 3β,16α-dihydroxy-pregn-5-en-20-one with potassium borohydride (in methanol–water), and separation of both isomers using solvent system VI (ratio 20α/20β, *ca.* 25:1).

Germany), using 20 × 20 cm glass plates; the 250 μ thick layers were prepared by use of the apparatus for thin-layer chromatography of Desaga. In all the experiments the starting line was 2.5 cm from the lower edge of the plate and at least 2 cm from the lateral border. The chromatogram was run in completely saturated tanks until the solvent front reached 15 cm from the starting line.

For the detection of the steroids, the following reactions were employed: (a) as a general reaction, a 1% solution of anisaldehyde in acetic acid–sulphuric acid (2:98); (b) for ketonic steroids, Gornall-McDonald reagent (0.1% 2,4-dinitrophenylhydrazine in a 10% ethanolic solution of hydrochloric acid); (c) for 17α,21-dihydroxy-20-ketonic steroids, Porter and Silber-reagent (phenylhydrazine solution in ethanolic sulphuric acid). A detailed description of these reactions was given previously[11]. The colours produced by the anisaldehyde reaction with the Δ^5-3-hydroxy-C_{21}-steroids investigated here are listed in Table I. As in previous studies[5–10], the following solvent systems were employed: (I) cyclohexane–ethyl acetate–ethanol (45:45:10), (II) ethyl acetate–*n*-hexane-glacial acetic acid–ethanol (72:13.5:19:4.5), (III) benzene–ethanol (40:10), (IV) benzene–ethanol (90:10), (V) chloroform–ethanol (90:10), (VI) chloroform–ethanol (95:5), (VII) ethyl acetate–cyclohexane (50:50), (VIII) ethyl acetate–*n*-hexane–glacial acetic acid (75:20:5), (IX) *n*-butanol–*tert.*-butanol–water (1:1:1) (upper phase).

Two special techniques were employed here for the separation of steroids of similar polarity ("critical pairs"): (a) Boric acid-impregnated layers: the silica gel layers were prepared by a mixture of 50 g of Silica Gel G with 100 ml of a 10% aqueous solution of boric acid; the plates were dried at room temperature overnight and heated for 30 min at 100° before use. (b) Isonicotinic acid hydrazone formation by elatography[10,12]. Using a capillary pipette, a 1.5 cm wide band on the starting line was treated with a 0.5% solution of isonicotinic acid hydrazine in a 10% acetic acid solution. Before this band was completely dry, the steroids were deposited on the starting line and allowed to stand for 1 h at room temperature. The hydrazones were then submitted to chromatography in solvent system IX.

RESULTS AND DISCUSSION

Tables II and III summarize the R_F and R_S (S = pregnenolone) values formed for the Δ^5-3-hydroxy-C_{21}-steroids investigated in this work in eight solvent systems.

By comparing the R_F values obtained in these systems for monosubstituted pregnolone derivatives, the following order of mobility was observed: 17α-ol > 21-ol > 16α-ol > 15α-ol > 7α-ol.

In all the solvent systems the order of polarity for 20-epimeric steroids not substituted at C-16 was 20α-ol > 20β-ol. However, for 16α-hydroxylated steroids, in both 3α- and 3β-hydroxy-Δ^5-pregnene series, the 20α-epimer moves faster than the corresponding 20β-epimer. The behaviour of these steroids on silica gel layers confirms the observation of HIRSCHMANN *et al.*[13,14] concerning the mobilities of pregnane-3,16α,20-triol isomers on paper chromatography. As these authors have pointed out, on 20α,16α-dihydroxysteroids, the formation of intramolecular hydrogen bonding could be expected and consequently a steroid with this structure moves faster than the corresponding 20β,16α-epimer.

The solvent systems presented here are suitable for the separation of Δ^5-pregnene-3,16α,20-triols from their isomeric Δ^5-pregnane-3,17α,20-triols. As a rule,

TABLE II

R_F AND R_S VALUES (S = 21-HYDROXY-PREGNENOLONE) AND THEIR STANDARD DEVIATIONS (SD) OBTAINED FOR THIRTY 3-HYDROXY-Δ^5-PREGNANE STEROIDS IN SOLVENT SYSTEMS I, II, III AND IV, WITH ASCENDIN ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G (MERCK AG, DARMSTADT).

(n is the number of experiments, and S.D. indicates the standard deviation of a single experiment. For the chromatographic conditions, see MATERIAL AND METHODS

Steroids	*Solvent systems*							
	I					*II*		
	n	R_F	*S.D.*	R_S	*S.D.*	*n*	R_F	*S.D.*
3β-ol-P^5-20-one	10	0.54	0.02	1.13	0.06	14	0.72	0.03
3β-ol-P^{5,16}-20-one	12	0.53	0.04	1.12	0.07	16	0.71	0.03
3β,7α-ol-P^5-20-one	11	0.26	0.02	0.58	0.05	9	0.44	0.03
3β,15α-ol-P^5-20-one	10	0.37	0.04	0.77	0.06	8	0.51	0.02
3β,16α-ol-P^5-20-one	8	0.35	0.04	0.74	0.06	20	0.52	0.03
3β,17α-ol-P^5-20-one	16	0.51	0.03	1.06	0.05	15	0.70	0.04
3β,21-ol-P^5-20-one	50	0.48	0.03			45	0.63	0.04
3β,17α,20α-ol-P^5-11-one	5	0.44	0.04	0.96	0.06	5	0.66	0.04
3β,11β, 17α-ol-P^5-20-one	5	0.34	0.03	0.74	0.05	6	0.51	0.03
3β,16α,17α-ol-P^5-20-one	7	0.35	0.02	0.74	0.05	8	0.52	0.03
3β,16α,17α-ol-P^5-20-one, 16α-Ac	5	0.45	0.02	1.00	0.05	6	0.70	0.03
3β,17α,21-ol-P^5-20-one	23	0.35	0.03	0.75	0.06	10	0.61	0.03
3β,17α,21-ol-P^5-20-one, 21-Ac	5	0.50	0.02	1.11	0.05	14	0.72	0.03
3β-ol-P^5-15,20-one	9	0.44	0.04	0.92	0.06	8	0.60	0.03
3β,11α-ol-P^5-7,20-one	6	0.20	0.02	0.42	0.04	6	0.30	0.03
3β-ol-P^5	12	0.64	0.02	1.34	0.09	10	0.79	0.03
3β,20α-ol-P^5	8	0.50	0.03	1.05	0.08	13	0.66	0.04
3β,20β-ol-P^5	10	0.53	0.03	1.11	0.08	14	0.69	0.04
3β,15α,20α-ol-P^5	9	0.19	0.03	0.42	0.05	8	0.35	0.02
3β,15α,20β-ol-P^5	9	0.30	0.04	0.63	0.04	8	0.48	0.02
3α,16α,20α-ol-P^5	9	0.30	0.03	0.63	0.04	8	0.42	0.03
3α,16α,20β-ol-P^5	10	0.27	0.02	0.56	0.04	7	0.41	0.03
3β,16α,20α-ol-P^5	6	0.30	0.02	0.66	0.05	6	0.46	0.03
3β,16α,20β-ol-P^5	6	0.25	0.01	0.55	0.04	6	0.44	0.03
3β,17α,20α-ol-P^5	8	0.38	0.04	0.84	0.06	9	0.57	0.02
3β,17α,20β-ol-P^5	8	0.41	0.04	0.86	0.06	15	0.61	0.03
3β,18,20β-ol-P^5	14	0.41	0.04	0.91	0.05	8	0.58	0.04
3β,20α,21-ol-P^5	8	0.26	0.03	0.58	0.04	6	0.46	0.02
3β,20β,21-ol-P^5	8	0.29	0.03	0.65	0.04	6	0.48	0.03
3β,11β,17α,20α-ol-P^5	5	0.29	0.03	0.63	0.05	5	0.51	0.03

the 16α-pregnene triols are more polar on silica gel layers than their 17α-isomers, as was similarly observed on paper chromatography by FUKUSHIMA *et al.*[15]

Steroids presenting epimerism at C-3 in the Δ^5-3-hydroxypregnane series are resolved in systems of chloroform–ethanol or ethyl acetate–cyclohexane type. In systems of chloroform–ethanol type the C_{21}-steroid with a 3α-hydroxy-Δ^5-structure moves slower than their 3β-epimer. In systems of benzene–ethanol type and in acidic systems (IV and V) one inversion of their mobility was observed. Similar inversion of polarity was found in such systems for steroids of the C_{19}-series, as 3α- and 3β-hydroxy-androst-5-en-17-one[7].

By comparing the results presented in this work with results obtained for the saturated pregnane steroids[8] it was found that in most of the systems employed, a valuable separation was achieved for the steroid fields presenting a 5βP-3α-ol/Δ^5P-3β-

		III					IV				
R_S	S.D.	n	R_F	S.D.	R_S	S.D.	n	R_F	S.D.	R_S	S.D.
1.20	0.07	14	0.56	0.04	1.12	0.06	15	0.42	0.03	1.27	0.07
1.16	0.07	13	0.55	0.03	1.12	0.08	12	0.42	0.03	1.25	0.08
0.71	0.03	8	0.41	0.02	0.76	0.02	6	0.22	0.03	0.64	0.05
0.81	0.03	8	0.46	0.04	0.88	0.06	6	0.23	0.02	0.64	0.04
0.82	0.06	15	0.47	0.04	0.91	0.04	12	0.26	0.02	0.75	0.03
1.12	0.06	20	0.53	0.04	1.02	0.05	20	0.35	0.03	0.98	0.05
		45	0.52	0.04			45	0.36	0.04		
1.01	0.06	5	0.46	0.03	0.87	0.04	5	0.31	0.02	0.82	0.03
0.79	0.04	6	0.40	0.03	0.76	0.03	5	0.23	0.02	0.61	0.03
0.87	0.04						8	0.25	0.03	0.73	0.03
1.17	0.06						8	0.33	0.02	0.93	0.05
0.98	0.04	8	0.43	0.03	0.82	0.03	10	0.25	0.03	0.70	0.03
1.18	0.07	10	0.55	0.03	1.07	0.05					
0.95	0.04	8	0.54	0.03	1.04	0.04	8	0.40	0.02	1.11	0.04
0.53	0.03						8	0.14	0.02	0.39	0.03
1.26	0.06	13	0.65	0.03	1.25	0.08	6	0.50	0.02	1.40	0.11
1.06	0.07	12	0.49	0.05	0.95	0.04	10	0.33	0.03	0.95	0.04
1.10	0.06	12	0.50	0.04	1.01	0.05	10	0.34	0.03	1.00	0.05
0.55	0.02	8	0.32	0.02	0.62	0.03	8	0.10	0.01	0.28	0.02
0.76	0.03	8	0.38	0.02	0.73	0.03	8	0.17	0.01	0.45	0.03
0.69	0.03	6	0.42	0.02	0.81	0.02	6	0.19	0.03	0.54	0.04
0.65	0.04	7	0.38	0.01	0.73	0.02	6	0.16	0.03	0.44	0.03
0.75	0.04	8	0.43	0.02	0.83	0.02	6	0.22	0.01	0.60	0.03
0.71	0.04	8	0.36	0.02	0.69	0.04	6	0.14	0.02	0.39	0.02
0.94	0.04	6	0.44	0.02	0.85	0.04	8	0.23	0.02	0.63	0.03
1.00	0.07	10	0.47	0.04	0.91	0.04	12	0.30	0.02	0.82	0.03
0.98	0.06	7	0.44	0.04	0.85	0.05	8	0.22	0.03	0.63	0.04
0.73	0.02	6	0.36	0.01	0.70	0.02	6	0.15	0.01	0.44	0.03
0.79	0.05	6	0.40	0.01	0.77	0.03	6	0.18	0.02	0.49	0.05
0.78	0.04	5	0.36	0.01	0.68	0.02	5	0.19	0.02	0.50	0.03

ol and 5βP-3β-ol/Δ^5P-3β-ol structure. However, as in the C_{19}-series[7], no resolution was achieved for steroid pairs having the structure 5αP-3β-ol/Δ^5-3β-ol. For the resolution of these "critical pairs", it is necessary to modify the structure of the unsaturated steroid. By treatment with perbenzoic acid, steroids with a Δ^5-3-hydroxy group form much more polar 5,6α- (and 5,6β-) oxido-derivatives, but the saturated steroid remains unchanged. This reaction has been employed for C_{19}-steroids with a Δ^5-3β-ol (ref. 16) and Δ^5-3α-ol (ref. 17) structure as well as for Δ^5-3β-hydroxy-C_{27}-sterols[18].

Unfortunately, several "critical pairs" of Δ^5-3-hydroxy-C_{21}-steroids have not been satisfactorily separated on silica gel layers by using the solvent systems employed here. For the resolution of these steroid pairs, the formation of derivatives or the application of special thin-layer chromatographic techniques were used.

As can be seen from the data presented in Tables II and III and those published

TABLE III

R_F AND R_S VALUES (S = 21-HYDROXY-PREGNENOLONE) AND THEIR STANDARD DEVIATIONS (S.D.) OBTAINED FOR THIRTY-ONE 3-HYDROXY-Δ^5-PREGNANE STEROIDS IN SOLVENT SYSTEMS V, VI, VII AND VIII, WITH ASCENDING ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G

For further particulars, see legend to Table II.

Steroids	*Solvent systems*							
	V					*VI*		
	n	R_F	*S.D.*	R_S	*S.D.*	*n*	R_F	*S.D.*
3β-ol-P^5-20-one	11	0.58	0.03	1.20	0.06	10	0.48	0.08
3β-ol-P^{5,16}-20-one	19	0.57	0.03	1.17	0.05	16	0.49	0.04
3β,7α-ol-P^5-20-one	14	0.31	0.02	0.61	0.04	12	0.12	0.01
3β,7α-ol-P^5-20-one, diAc	6	0.75	0.03	1.54	0.07			
3β,15α-ol-P^5-20-one	8	0.38	0.03	0.78	0.06	10	0.17	0.02
3β,16α-ol-P^5-20-one	12	0.41	0.03	0.80	0.03	10	0.21	0.02
3β-17α-ol-P^5-20-one	24	0.50	0.08	1.06	0.05	19	0.33	0.03
3β,21-ol-P^5-20-one	55	0.52	0.04			52	0.36	0.03
3β,17α-20α-ol-P^5-11-one	5	0.37	0.03	0.66	0.05	5	0.13	0.01
3β,11β,17α-ol-P^5-20-one	5	0.31	0.03	0.55	0.05	5	0.11	0.01
3β,16α,17α-ol-P^5-20-one	6	0.47	0.02	0.89	0.04	6	0.26	0.02
3β,16β,17β-ol-P^5-20-one, 16αAc	6	0.60	0.03	1.13	0.05	6	0.42	0.02
3β,17α,21-ol-P^5-20-one	14	0.34	0.03	0.68	0.04	10	0.17	0.02
3β,17α,21-ol-P^5-20-one, 21-Ac	12	0.51	0.03	1.04	0.05			
3α-ol-P^5-15,20-one	8	0.51	0.02	1.07	0.04	10	0.37	0.02
3β,11β-ol-P^5-7,20-one	6	0.19	0.02	0.36	0.03	7	0.05	0.01
3β-ol-P^5	12	0.61	0.03	1.25	0.06	10	0.50	0.03
3β,20α-ol-P^5	12	0.48	0.03	0.95	0.04	10	0.33	0.03
3β,20β-ol-P^5	13	0.50	0.04	1.04	0.05	10	0.36	0.03
3β,15α,20α-ol-P^5	8	0.15	0.01	0.29	0.02	8	0.06	0.01
3β,15α,20β-ol-P^5	8	0.28	0.03	0.54	0.05	8	0.12	0.01
3α,16α,20α-ol-P^5	8	0.32	0.01	0.61	0.02	7	0.11	0.01
3α,16α,20β-ol-P^5	8	0.22	0.01	0.41	0.04	7	0.08	0.01
3β,16α,20α-ol-P^5	6	0.38	0.02	0.74	0.05	5	0.15	0.01
3β,16α,20β-ol-P^5	6	0.26	0.01	0.52	0.06	5	0.10	0.01
3β,17α,20α-ol-P^5	10	0.36	0.04	0.72	0.05	8	0.14	0.02
3β,17α,20β-ol-P^5	16	0.40	0.04	0.78	0.05	20	0.22	0.03
3β,18,20β-ol-P^5	7	0.36	0.02	0.69	0.04	11	0.18	0.02
3β,20α,21-ol-P^5	7	0.24	0.02	0.49	0.04	8	0.10	0.01
3β,20β,21-ol-P^5	8	0.26	0.03	0.54	0.06	10	0.10	0.02
3β,11β,17α,20α-ol-P^5	5	0.21	0.02	0.37	0.03	5	0.06	0.01

by other authors[19,20], the introduction of an unsaturated bond at C_{16} does not lead to a significant change of the mobility of the "root" steroid. Neither on untreated silica gel layers[3,19,21], nor on aluminum oxide[21], nor on magnesium silicate layers[20] could a mixture of pregnenolone and 16-dehydropregnenolone be resolved. Identical difficulty was encountered in the attempted separation of progesterone–16-dehydroprogesterone on aluminum oxide[21] and in silica gel layers[22], as well as 16-dehydro–16-dihydro-C_{19}-steroids on untreated silica gel layers[23,24].

Likewise, as indicated for the pair progesterone–16-dehydroprogesterone[8], the steroids pregnenolone and 16-dehydropregnenolone also can be separated after elatographic treatment with isonicotinic acid hydrazine in diluted acetic acid. In contrast to α,β-unsaturated and isolated ketones, which react immediately or in a few minutes with the formation of isonicotinic acid hydrazones, the Δ^{16}-20-ketonic function

		VII					*VIII*				
R_S	S.D.	*n*	R_F	S.D.	R_S	S.D.	*n*	R_F	S.D.	R_S	S.D.
1.30	0.06	11	0.37	0.03	1.88	0.10					
1.31	0.07	10	0.37	0.03	1.82	0.15					
0.34	0.04						6	0.24	0.02	0.47	0.05
		7	0.47	0.02	2.43	0.15	3	0.71	0.03	1.56	0.08
0.51	0.06	8	0.08	0.01	0.43	0.04	6	0.36	0.02	0.70	0.03
0.59	0.04	10	0.09	0.01	0.45	0.04	5	0.38	0.02	0.83	0.03
0.96	0.04	14	0.28	0.03	1.40	0.09	6	0.58	0.03	1.22	0.03
		30	0.19	0.02			18	0.48	0.03		
0.37	0.03	5	0.13	0.01	0.62	0.05	5	0.49	0.03	0.92	0.05
0.31	0.03	5	0.07	0.01	0.33	0.03	5	0.33	0.02	0.62	0.03
0.74	0.04	5	0.12	0.01	0.54	0.04	6	0.44	0.02	0.90	0.04
1.10	0.04	5	0.23	0.02	1.05	0.06	6	0.53	0.03	1.06	0.06
0.46	0.04	17	0.14	0.02	0.72	0.05					
		25	0.53	0.03	2.80	0.18					
1.09	0.05	6	0.16	0.02	0.82	0.06	6	0.46	0.03	0.96	0.03
0.12	0.01										
1.38	0.07	9	0.51	0.03	2.48	0.11					
0.89	0.03	8	0.27	0.02	1.30	0.10					
0.98	0.03	8	0.31	0.03	1.51	0.09					
0.13	0.02	7	0.02	0.004	0.15	0.02	7	0.15	0.01	0.31	0.02
0.32	0.03	7	0.06	0.01	0.30	0.04	7	0.29	0.02	0.62	0.02
0.30	0.01	8	0.02	0.007	0.11	0.02	7	0.26	0.03	0.52	0.05
0.15	0.01	8	0.02	0.008	0.10	0.01	9	0.19	0.02	0.38	0.03
0.43	0.03	6	0.14	0.01	0.74	0.05	6	0.27	0.02	0.55	0.04
0.22	0.02	6	0.08	0.01	0.42	0.04	6	0.24	0.02	0.51	0.04
0.42	0.05	7	0.11	0.01	0.60	0.06	7	0.40	0.02	0.85	0.04
0.61	0.03	7	0.16	0.01	0.87	0.06	7	0.43	0.03	0.98	0.04
0.44	0.03	8	0.11	0.01	0.51	0.05					
0.28	0.03	6	0.05	0.01	0.26	0.04	5	0.28	0.02	0.60	0.03
0.29	0.03	6	[illegible]	0.01	0.35	0.05	5	0.31	0.02	0.56	0.03
0.17	0.01	5	0.05	0.01	0.24	0.03	5	0.31	0.02	0.58	0.03

reacts very slowly. Fig. 1 shows the elatograms of progesterone, 16-dehydroprogesterone, pregnenolone and 16-dehydropregnenolone, developed with solvent system IX. It can be seen that under the experimental conditions employed for the reaction, pregnenolone forms a 20-hydrazone, whereas 16-dehydropregnenolone remains unchanged. Under the same conditions, 16-dehydroprogesterone gives a 3-monohydrazone, whereas progesterone and 6-dehydroprogesterone show the formation of a 3 and/or 20-monohydrazone(s) and a 3,20-diisonicotinic acid hydrazone.

On boric acid-impregnated silica gel layers some pregnanetriols move faster than on untreated silica gel layers[25]. Because glycols having a 17α,20β-structure form weak polar complexes with boric acid, a better separation is obtained for the epimeric Δ^5-pregnane-3β,17α,20β-/3β,17α,20α-triols on such layers.

At the same time, although only the 20β-epimer is able to form the less polar

cycloborates[26], even the steroids with a 17α,20α group migrate faster on boric acid–silica gel than on silica gel layers. Therefore, this method also allows a better separation between 17α,20-glycol-steroids and those steroids whose migration using solvent systems I, IV, VI and VII is not changed on boric acid–silica gel layers, such as Δ^5-pregnane-3β,15α,20β-triol, Δ^5-pregnane-3α,16α,20β-triol and Δ^5-pregnane-3β,16α,20β-

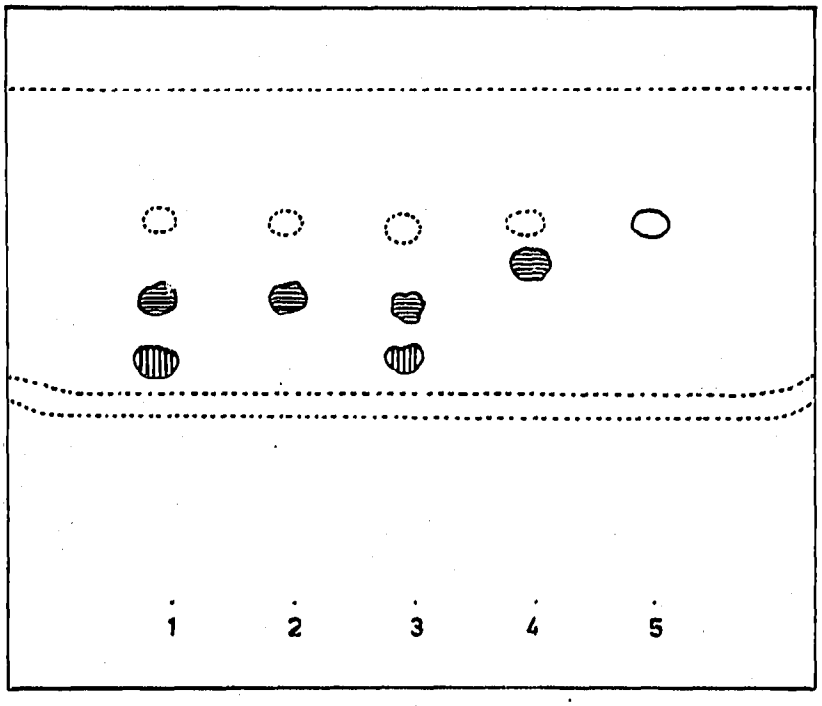

Fig. 1. Elatography of progesterone (1), 16-dehydroprogesterone (2), 6-dehydroprogesterone (3), pregnenolone (4) and 16-dehydropregnenolone (5) on isonicotinic acid hydrazine treated silica gel layers. In contrast to progesterone and 6-dehydroprogesterone, 16-dehydroprogesterone gives only a 3-monohydrazone derivative. Pregnenolone, but not 16-dehydropregnenolone forms isonicotinic acid hydrazone. For the experimental conditions see text. Shadow spots: monohydrazone (horizontally ruled), dihydrazone (vertically ruled). Mobility of isonicotinic acid hydrazine: 5.8 cm (front: 15 cm).

TABLE IV

CHROMATOGRAPHIC MOBILITIES (R_F × 100 VALUES) OBTAINED FOR Δ^5-PREGNENETRIOLS WITH ASCENDING ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY ON SILICA GEL–BORIC ACID LAYERS

Each value is the mean value obtained from three chromatograms. For the experimental conditions and solvent systems, see MATERIALS AND METHODS.

Steroids	*Solvent systems*					
	I	*II*	*IV*	*VI*	*VII*	*VIII*
3β,15α,20β-ol-P[5]	33	49	14	15	4	28
3α,16α,20β-ol-P[5]	30		17	12	6	16
3β,16α,20α-ol-P[5]	40		31	28	15	29
3β,16α,20β-ol-P[5]	28		15	15	11	22
3β,17α,20α-ol-P[5]	51	58	45	50	29	44
3β,17α,20β-ol-P[5]	56	62	46	51	35	46
3β,20α,21-ol-P[5a]	43		36		16	35
3β,20β,21-ol-P[5a]	47		40		18	37

[a] Obtained by courtesy of Prof. E. DICZFALUSY.

triol (Table IV). It is of interest to note that contrary to Δ^5-pregnene-3β,16α,20β-triol, its 20α-epimer forms a borate-complex and a good separation between both isomers can be achieved on boric acid-treated layers.

Only a partial separation could be achieved between Δ^5-pregnene-3β,20α- and 3β,20β-diols. A complete resolution of these isomers can be accomplished by specific oxidation of the 20β-hydroxyl group with 20β-hydroxysteroid oxidoreductase[27]. The enzymatic-formed pregnenolone separates easily from the unchanged 20α-epimer.

ACKNOWLEDGEMENTS

For a generous supply of the steroids used in this work, the author is greatly indebted to Dr. A. A. AKHREM (U.S.S.R.), Dr. S. BERNSTEIN (U.S.A.), Dr. O. A. DE BRUIN (The Netherlands), Dr. R. I. COX (Australia), Prof. E. DICZFALUSY (Sweden), Dr. M. FINKELSTEIN (Israel), Dr. D. FUKUSHIMA (U.S.A.), Dr. J. JOSKA (Czechoslovakia), Prof. K. JUNKMANN (Germany), Prof. W. KLYNE (England), Dr. L. KOGAN (U.S.S.R.), Dr. G. SNATZKE (Germany) and Dr. L. STARKA (Czechoslovakia).

Part of the expenses of this investigation was defrayed by a grant from the Swedish Medical Research Council (B69-13X-2525-01) and from "Stiftelsen Therèse and Johan Anderssons Minne".

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