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1 J. AVIGAN, D. S. GOODMAN ET D. STEINBERG, *J. Lipid Res.*, 4 (1963) 100.
2 J. D. JOHNSTON, F. GAUTSCHI ET K. BLOCH, *J. Biol. Chem.*, 224 (1957) 185.
3 C. B. BARRET, M. S. J. DALLAS ET F. B. PADLEY, *Chem. Ind. (London)*, (1962) 1050.
4 E. HAAHTI ET T. NIKKARI, *Acta Chem. Scand.*, 17 (1963) 536.
5 L. J. MORRIS, *J. Lipid. Res.*, 4 (1963) 357.
6 J. R. CLAUDE ET J. L. BEAUMONT, *Ann. Biol. Clin. (Paris)*, 22 (1964) 815.

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R_F values of some estrogens and 3β-hydroxy-Δ⁵-steroids in thin-layer chromatography without binder

Recently, much attention has been paid to the biological significance of 3β-hydroxy-Δ^5-steroids as the precursors of estrogen formation in the ovary and placenta. Many attempts have been made to separate estrogens, Δ^5-androstene and Δ^5-pregnene derivatives. The use of thin-layer chromatography on silica gel was systematically studied for this purpose and excellent results were obtained[1,2]. In the present communication the chromatographic technique for the separation of the most important naturally occurring estrogens and 3β-hydroxy-Δ^5-steroids on a thin layer of alumina without binder[3] is described.

Alumina without binder (activity III for 3β-hydroxy-Δ^5-steroids and activity IV for estrogens, 200–250 mesh) was freely spread on a glass plate (12 × 22 cm) and a layer 10 cm wide and 0.6–0.8 mm thick was smoothed by means of a glass rod with polythene tubing sleeves as described previously[4]. Steroid samples in chloroform were spotted on the start-line and the chromatogram was developed by the ascending technique at a slope of 15° in a chromatographic tank completely saturated with the mobile phase poured into the bottom. The solvent front reached the upper end of the glass plate within 25–30 min.

3β-Hydroxy-Δ^5-steroids were detected by spraying the plate after drying with ALLEN's reagent[5] (80 ml conc. sulphuric acid and 20 ml 90% ethanol); the purple spots appeared without heating. 7-Hydroxy-Δ^5-steroids and $\Delta^{5,7}$-dienes gave an azure-blue coloration. The sensitivity of the reaction was 1-2 μg per spot. Estrogens were detected by spraying the surface of the chromatogram while still moist with ferricyanide–ferric chloride reagent[6].

TABLE I

THE R_F VALUES (× 100) OF SOME 3β-HYDROXY-Δ[5]-STEROIDS IN THIN-LAYER CHROMATOGRAPHY ON ALUMINA (ACTIVITY III) WITHOUT BINDER*

Steroid	*$CHCl_3$–EtAc*		*$CHCl_3$–EtOH*	*$CHCl_3$–EtAc-EtOH*				*CH_2Cl_2–EtAc*		*C_6H_6–EtAc–EtOH*	*C_6H_6–EtOH*
	(90:10)	(93:7)	(97:3)	(87:12:1)	(89:7:4)	(90:7:2)	(92:7:1)	(80:20)	(90:10)	(86:10:4)	(96:4)
Δ[5]-Androstene-3β,7α,17β-triol	2	1	27	4		9	8			4	3
Δ[5]-Pregnene-3β,17α,21-triol-20-one	2	2	32	5		12	9			8	5
Δ[5]-Androsten-3β-ol-7,17-dione	2	2	36	6		11	10			9	5
Δ[5]-Androstene-3β,7α-diol-17-one	3	3	36	7	41	17	11	4	2	7	5
Δ[5]-Pregnene-3β,17α,20α-triol	6	5	50	11		24	19			13	10
Δ[5]-Androstene-3β,16α-diol-17-one	13	11	57	21	60	33	30	16	8	19	13
Δ[5]-Pregnene-3β,21-diol-20-one	16	15	59	27		37	32			22	18
Δ[5]-Androstene-3β,17β-diol	26	25	59	38		44	40			28	19
Δ[5]-Pregnene-3β,17α-diol-20-one	29	27	64	39		45	42	44	25	30	20
Δ[5]-Pregnene-3β,20α-diol	29	28	64	39	70	50	48	50	27	32	22
Δ[5,7]-Androstadien-3β-ol-17-one				40		52	49			33	24
Δ[5]-Androsten-3β-ol-17-one	54	48	68	53	77	53	52	70	50	40	30
Δ[5]-Pregnen-3β-ol-20-one	68	60	69	55	79	60	58	73	53	42	35

* $CHCl_3$ = chloroform (without ethanol); CH_2Cl_2 = dichloromethane; C_6H_6 = benzene; EtOH = abs. ethanol; EtAc = ethyl acetate.

TABLE II

THE R_F VALUES ($\times$ 100) OF SOME ESTROGENS IN THIN-LAYER CHROMATOGRAPHY ON ALUMINA (ACTIVITY IV) WITHOUT BINDER*

Estrogen	*EtAc*	*EtEther*	*AmAc*	*CCl_4-MeOH*			*CCl_4-EtOH*		*CCl_4-EtAc*	*CCl_4-PrOH*			*$CHCl_3$-MeOH*	
				(85:15)	(90:10)	(95:5)	(85:15)	(90:10)	(50:50)	(85:15)	(90:10)	(95:5)	(90:10)	(95:5)
Estrone	77	96	90	35	32	22	84	63	44	front	74	44	80	77
Estradiol-17β	57	85	84	26	22	16	74	48	28	89	63	31	80	63
Estriol	4	9	23	10	8	2	33	18	2	21	12	2	46	14
16-*epi*-Estriol	8	33	45	15	10	6	39	25	4	28	18	7	55	27
6α-Hydroxy-estradiol-17β	5	31	51	8	5	3	37	19	3	24	14	4	49	18
16-Oxo-estradiol-17β	24	48	68	22	20	12	64	41	10	66	44	19	77	54

Estrogen	*$CHCl_3$-EtOH*		*$CHCl_3$-EtAc*	*$CHCl_3$-PrOH*	*CH_2Cl_2-MeOH*		*CH_2Cl_2-EtOH*		*CH_2Cl_2-EtAc*	*CH_2Cl_2-PrOH*		*CH_2Cl_2-AmAc*	
	(90:10)	(95:5)	(90:10)	(95:5)	(90:10)	(95:5)	(90:10)	(95:5)	(90:10)	(90:10)	(95:5)	(75:25)	(50:50)
Estrone	88	80	35	78	front	70	95	76	38	95	81	70	73
Estradiol-17β	87	67	21	63	front	57	90	67	14	92	68	62	68
Estriol	33	11	1	6	67	7	32	14	1	20	7	6	10
16-*epi*-Estriol	49	30	3	18	75	18	47	32	1	40	17	19	23
6α-Hydroxy-estradiol-17β	40	15	1	9	68	9	42	20	1	31	11	7	14
16-Oxo-estradiol-17β	85	58	11	53	92	51	87	64	7	92	61	48	51

Estrogen	*$C_2H_3Cl_3$-MeOH*	*$C_2H_3Cl_3$-PrOH*	*$C_2H_4Cl_2$-MeOH*		*$C_2H_4Cl_2$-PrOH*	*C_6H_6-MeOH*		*C_6H_6-EtOH*		*C_6H_6-PrOH*	
	(95:5)	(95:5)	(90:10)	(95:5)	(95:5)	(90:10)	(95:5)	(90:10)	(95:5)	(90:10)	(95:5)
Estrone	29	58	68	65	64	48	25	51	32	76	57
Estradiol-17β	18	44	54	38	45	38	17	45	22	68	44
Estriol	3	3	28	6	4	18	2	22	2	14	4
16-*epi*-Estriol	7	12	36	13	11	25	6	25	6	21	8
6α-Hydroxy-estradiol-17β	5	4	25	8	7	19	4	23	4	18	5
16-Oxo-estradiol-17β	15	30	50	30	38	34	13	39	16	62	29

* CCl_4 = carbon tetrachloride; C_6H_6 = benzene; $C_2H_3Cl_3$ = trichloroethylene; $C_2H_4Cl_2$ = 1,2-dichloroethylene; CH_2Cl_2 = dichloromethane; $CHCl_3$ = chloroform; MeOH = methanol; EtOH = ethanol; PrOH = *n*-propanol; EtAc = ethyl acetate; AmAc = amyl acetate; EtEther = diethyl ether.

The R_F values in various solvent systems composed of halogenated hydrocarbons or benzene with the addition of alcohol, an ester or ether are listed in Tables I and II. Substitution of the estratrien, Δ^5-androstene and Δ^5-pregnene nucleus influences the R_F values in the usual manner as seen in adsorption chromatography on alumina. In estrogens, the mobility is decreased by the functional groups in the sequence: 16-ketone $<$ 16β-hydroxyl $<$ 6α-hydroxyl $<$ 16α-hydroxyl and in the 3β-hydroxy-Δ^5-steroids: Δ^7-double bond $<$ 17α-hydroxyl $<$ 16α-hydroxyl $<$ 21 hydroxyl $<$ 7-ketone $\leqslant$ 7α-hydroxyl.

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1 B. P. LISBOA AND E. DICZFALUSY, *Acta Endocrinol.*, 40 (1962) 60.
2 B. P. LISBOA, *J. Chromatog.*, in press.
3 M. MOTTIER AND M. POTTERAT, *Anal. Chem. Acta*, 13 (1955) 46.
4 L. STÁRKA AND J. MALÍKOVÁ, *J. Endocrinol* , 22 (1961) 215.
5 W. M. ALLEN, S. J. HAYWARD AND A. PINTO, *J. Clin. Endocrinol. and Metab.*, 10 (1950) 54.
6 G. M. BARTON, R. S. EVANS AND J. A. F. GARDNER, *Nature*, 170 (1952) 249.

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J. Chromatog., 17 (1965) 599–602

Dünnschichtchromatographie von Aminozuckern auf Cellulosepulver

In den letzten Jahren wurden einige Verfahren zur Dünnschichtchromatographie von Zuckern bekannt. Während hierbei zunächst anorganische Schichten wie Kieselgel oder Kieselguhr[1] Verwendung fanden, wurde Cellulosepulver erstmals von SCHWEIGER[2] zur Trennung von Monosacchariden eingeführt. Nach diesen Ergebnissen schien es möglich, auch substituierte Zucker wie Glucosamin und Galactosamin und deren Acetylderivate auf Celluloseschichten zu trennen. In der vorliegenden Mitteilung wird über Versuche hierzu berichtet.

Die Platten wurden in bekannter Weise[2] mit dem Streichgerät der Fa. Desaga (Heidelberg) mit Cellulosepulver MN 300 der Fa. Macherey und Nagel (Düren, Deutschland) beschichtet (Schichtdicke 0.25 mm). Folgende Laufmittelgemische hatten sich bewährt:

I.	Butanol–Äthanol–Isopropanol–Ammoniak–Wasser	(2:4:0.5:0.5:1.5)
II.	Pyridin–Äthylacetat–Eisessig–Wasser	(5:5:1:3)
III.	Äthanol–Pentanol–Ammoniak–Wasser	(8:2:2:1)
IV.	Äthylacetat–Pyridin–Tetrahydrofuran–Wasser	(7:3:2:2)
V.	Äthylacetat–Isopropanol–Pyridin–Wasser	(7:3:2:2)

Gemische IV und V wurden bei Celluloseschichten angewandt, die mit Boratpuffer von pH = 8.0 (0.2 *M* Borsäure, 0.05 *M* NaCl und 0.05 *M* Borax = $Na_2B_4O_7 \cdot 10\ H_2O$) besprüht worden waren. Die Entwicklung der Chromatogramme dauerte etwa 2–3 Std. Eine Trennung von Glucosamin, Galactosamin, N-Acetylglucosamin und N-Acetylgalactosamin war in Systemen I, II und IV möglich (Tabelle I).