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Thin-layer chromatography of C_{19}-steroid 2,4-dinitrophenylhydrazones on polyamide

Since the reaction of 2,4-dinitrophenylhydrazine with ketosteroids[1,2] has been shown to be quite useful for the qualitative and quantitative analyses of ketonic C_{19}-steroids[2-4], the isolation of corresponding derivatives by thin-layer chromatography (TLC) gained considerable interest. So far, however, the separation of epimeric C_{19}-steroid 2,4-dinitrophenylhydrazones by TLC on silica gel or alumina presented some difficulties, thus calling for the elaboration of new chromatographic systems.

By using reversed-phase TLC on polyamide and suitable solvent systems, adequate resolution of closely related C_{19}-steroid derivatives may be obtained. For such purposes, 5–25 μg of 2,4-dinitrophenylhydrazones are dissolved in chloroform and applied to the polyamide layer (0.1-mm thickness, Selecta-Fertigplatten Nr. 1600, Schleicher & Schüll, 3354 Dassel, G.F.R.) by means of a capillary syringe. Diffuse spots should be concentrated to a narrow band on the starting line by ascending chromatography in methylene chloride. Chromatograms are developed by the ascending technique in a paper-lined chamber filled with the mobile phase.

Table I contains the R_F values of numerous C_{19}-steroid 2,4-dinitrophenylhydrazones in different solvent systems. All R_F values represent the mean of at least three determinations. As can be seen, the derivatives of epimeric C_{19}-steroids such as 17β- or 17α-hydroxy-4-androsten-3-one (testosterone and epitestosterone), 3α- or 3β-hydroxy-5α-androstan-17-one (androsterone and epiandrosterone), 3α- or 3β-hydroxy-5β-androstan-17-one (etiocholanolone and epietiocholanolone) and 3α- or 3β-hydroxy-4-androsten-17-one (3α- and 3β-hydroxy-4-androstenone) are readily separated in every solvent system. By combination of reversed-phase TLC on poly-

TABLE I

THIN-LAYER CHROMATOGRAPHY OF C_{19}-STEROID 2,4-DINITROPHENYLHYDRAZONES ON POLYAMIDE

Solvent systems: 1 = methylene chloride–methanol (2:8); 2 = 1,2-dichloroethane–methanol (2:8); 3 = trichloroethylene–methanol (2:8); 4 = methylene chloride–methanol–water (5:7:3); 5 = trichloroethylene–methanol–water (20:64:16); 6 = acetone–methanol–acetic acid (20:80:0.5); 7 = carbon tetrachloride–acetone–methanol–water (5:10:40:10); 8 = acetone–ethanol–water (5:16:4).

Derivative of	*R_F value in solvent system*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Testosterone	0.22	0.30	0.34	0.16	0.25	0.17	0.09	0.11
Epitestosterone	0.47	0.53	0.53	0.33	0.39	0.39	0.23	0.28
Androsterone	0.65	0.69	0.66	0.50	0.52	0.55	0.40	0.53
Epiandrosterone	0.40	0.43	0.44	0.26	0.39	0.26	0.14	0.20
Etiocholanolone	0.71	0.77	0.72	0.62	0.61	0.68	0.54	0.72
Epietiocholanolone	0.66	0.71	0.67	0.54	0.56	0.60	0.43	0.56
3α-OH-4-Androstenone	0.53	0.67	0.64	0.49	0.57	0.67	0.39	0.57
3β-OH-4-Androstenone	0.67	0.56	0.55	0.37	0.54	0.51	0.23	0.38
11β-OH-Androsterone	0.64	0.66	0.65	0.53	0.60	0.67	0.44	0.59
11β-OH-Etiocholanolone	0.71	0.72	0.68	0.60	0.65	0.71	0.54	0.73
Dehydroepiandrosterone	0.38	0.41	0.42	0.24	0.36	0.26	0.14	0.19
3β-Chloro-dehydroepiandrosterone	0.66	0.68	0.65	0.48	0.57	0.66	0.38	0.58

amide with TLC on Silica Gel G in chloroform–dioxan (94:6)[5], the isolation of most C_{19}-steroid 2,4-dinitrophenylhydrazones becomes feasible, facilitating the estimation of these compounds in biological extracts[5,6]. At the same time, individual derivatives in discrete spots may be quantitated after TLC on polyamide by means of direct densitometry with a spectrodensitometer (Model SD 3000, Schoeffel Instr. Corp., Westwood, N.J., U.S.A.). The estimation of C_{19}-steroid 2,4-dinitrophenylhydrazones by this procedure, the sensitivity of which approximates 10 ng, as well as its application to the analysis of C_{19}-steroids in biological material, will be presented in a forthcoming communication.

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Rapid quantitation of Δ^4-3-ketosteroids by thin-layer densitometry

Thin-layer densitometry of steroids has recently received increased attention. Such methods are based either on staining of free substances after chromatography[1] or the formation of coloured derivatives[2–4]. The present paper describes the quantitation of Δ^4-3-ketosteroids by use of their quench effect upon the fluorescence at 254 mμ provided by a suitable dye in the adsorbent.

Methods

For chromatography Analtech (Wilmington, Del., U.S.A.) plates with a 250 μ thick layer were used. The coating material was Silica Gel GF_{254} with fluorescence at 254 mμ. In a special scoring device (Schoeffel Instr. Corp., Westwood, N.J., U.S.A.) the thin layer was divided into lanes of 1 cm width. Because of the double beam operating system of the densitometer only alternate lanes were loaded with a mixture of Δ^4-3-ketosteroids. The blank lanes served as reference for the instrument. The plates were then developed in a suitable solvent system such as chloroform–dioxane (94:6) yielding adequate separation of various steroids.

Direct quantitation was performed by means of a Schoeffel spectrodensitometer, Model SD 3000 (Schoeffel Instr. Corp., Westwood, N.J., U.S.A.). A quartz