CHROM. 5504

Steroids

I. Application of thin-layer chromatography to the separation of 2,4-dinitrophenylhydrazones of the physiological important 17-ketosteroids

The 11-desoxy-17-ketosteroids (etiocholanolone and androsterone) constitute the largest percentage of excreted metabolites of 17-ketosteroids (17-KS) in human urine. Their separation with dehydroepiandrosterone and 11-substituted 17-KS, mainly 11β-hydroxyetiocholanolone, 11β-hydroxyandrosterone, 11-oxoetiocholanolone and 11-oxoandrosterone, are most important from the medical view point. The literature concerning the thin-layer chromatography on silica gel suggests a number of methods for the chromatography of 2,4-dinitrophenylhydrazones of some 17-KS[1–7], but none of these systems is satisfactory for the separation of all the above steroid 2,4-dinitrophenylhydrazones from mixtures.

This paper describes the application of five solvent systems to the seven physiologically most important 17-KS after their reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH) and a two-dimensional method for their complete separation with a view to their later rapid determination in urine.

Experimental

Materials. Silica Gel G and Silica Gel $HF_{254+366}$ (Merck) were used as adsorbents. Sources, systematic and trivial names of the steroids studied are listed in Table I. The solvents used for development of the chromatograms were redistilled, chloroform contained 0.6–1.0 % ethanol.

General methods. The preparation of 2,4-dinitrophenylhydrazones of 17-KS was carried out by the method of TREIBER AND OERTEL[3]. One-dimensional chromatograms were run on 10 × 20 cm glass plates spread with a 0.25–0.30 mm layer of the silica gel mixture (Silica Gel G–Silica gel $HF_{254+366}$, 3:1). The plates were stored over-

TABLE I

SYSTEMATIC NAMES, TRIVIAL NAMES AND SOURCES OF THE SEVEN 17-KETOSTEROIDS STUDIED

No.	*Systematic name*	*Trivial name*	*Abbreviation*	*Source*[a]
1	3β-Hydroxyandrost-5-en-17-one	Dehydroepiandrosterone	3βolΔ^5A17one	a
2	3α-Hydroxy-5α-androstan-17-one	Androsterone	3αol5αA17one	a
3	3α-Hydroxy-5β-androstan-17-one	Etiocholanolone	3αol5βA17one	a
4	3α,11β-Dihydroxy-5α-androstan-17-one	11-Hydroxyandrosterone	3α,11βol5αA17one	b
5	3α,11β-Dihydroxy-5β-androstan-17-one	11-Hydroxyetiocholanolone	3α,11βol5βA17one	c
6	3α-Hydroxy-5α-androstan-11,17-dione	11-Oxoandrosterone	3αol5αA11,17one	b
7	3α-Hydroxy-5β-androstan-11,17-dione	11-Oxoetiocholanolone	3αol5βA11,17one	c

[a] a = Calbiochem, Los Angeles, U.S.A.; b = Sigma Chemical Corp., St. Louis, U.S.A.; c = [illegible] Laboratories, Colnbrook, Great Britain.

night open to the atmosphere. In the two-dimensional method glass plates 18 × 18 cm were used.

Application and sample development. The samples were applied as small spots 3 cm from the edge of the 10 × 20 cm plate and the immersion line was about 0.5 cm. In two-dimensional chromatography, the samples were applied 2 cm from the edge of the plate.

The atmophere inside a chromatographic tank, fitted with filter paper on two sides, was first equilibrated for 3 h with the solvent to be used for development. The solvent was allowed to ascend 16 cm. After the first development, the plate was dried for 5 min with a stream of air, then equilibrated for 5 min at room temperature, and the solvent was allowed to ascend the plate a second time in the same way.

The location of spots containing less than 1 μg of 2,4-dinitrophenylhydrazones was most easily performed by examination of the chromatogram in UV light of an appropriate wavelength, such as that obtained with the Narva HQV 125 (366 nm). The hydrazones then appeared as dark contrast spots on a light background.

Results and discussion

The following solvent systems were employed:
System A: chloroform—acetone (98:2)
System B: chloroform—acetone (96:4)
System C: chloroform—acetone (94:6)
System D: chloroform—acetone (92:8)
System E: diethyl ether—petroleum ether (70:30)

The chromatographic results for seven 2,4-dinitrophenylhydrazones of 17-KS separated by two initial runs in the same system are summarised in Fig. 1 and Table II.

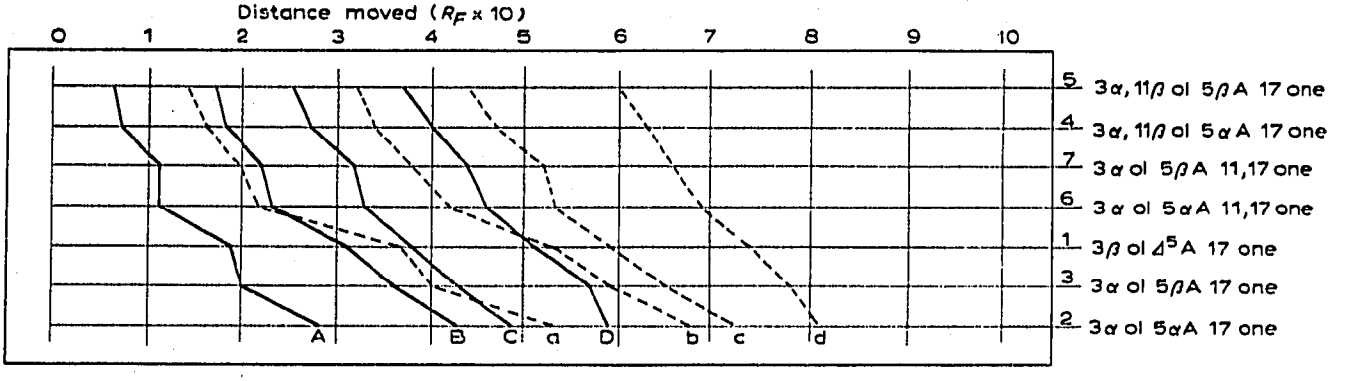


Fig. 1. Comparison of mobilities of 2,4-dinitrophenylhydrazones of 17-ketosteroids on silica gel. Solvents were chloroform–acetone in the ratios (A) 98:2; (B) 96:4, (C) 94:6; (D) 92:8. ——, after first development; ----- after second development.

By preliminary experiments with systems containing diethyl ether and petroleum ether in the proportions 60:40, 70:30 and 80:20, it was found that for the separation of 11-substituted 17-KS as hydrazones, the first two mixtures were preferable. Th[illegible] disadvantage of the first mixture was the small R_F values and therefore 70:30 [illegible]ture (system E) was used as the most convenient system.

[illegible]stem, with rechromatography, allowed the separation of all the phy[illegible] logi[illegible] [illegible]ortant 17-KS listed in Table II as 2,4-dinitrophenylhydrazones, [illegible] hor[illegible]

TABLE II

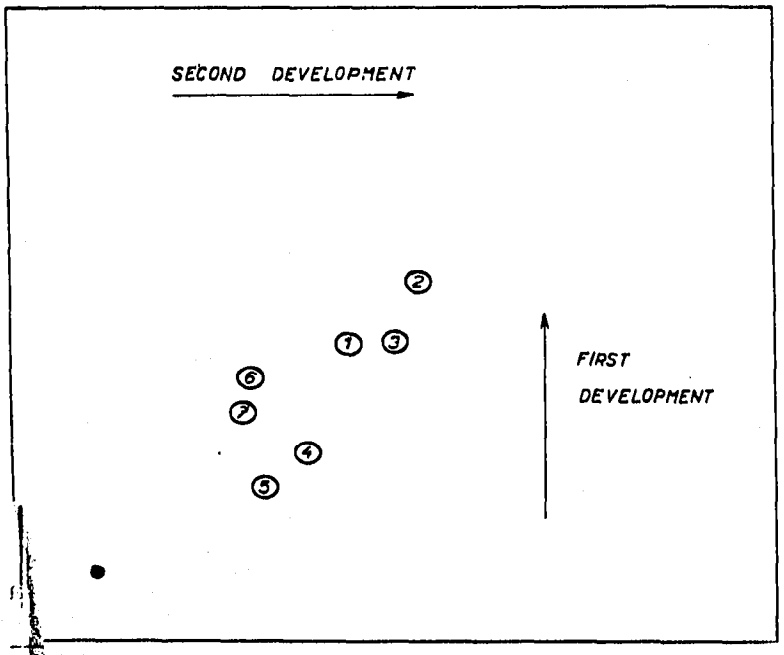
R_F OF SEVEN 17-KETOSTEROIDS IN SYSTEM E AFTER THE FIRST AND SECOND DEVELOPMENTS

No.	*Steroid (as 2,4-DNPH derivative)*	R_F	
		After first development	*After two developments*
1	3βolΔ^5A17one	0.28	0.44
2	3αol5αA17one	0.35	0.53
3	3αol5βA17one	0.30	0.45
4	3α11βol5αA17one	0.25	0.38
5	3α11βol5βA17one	0.21	0.33
6	3αol5αA11,17one	0.16	0.25
7	3αol5βA11,17one	0.12	0.20
8	2,4-DNPH[a]	0.09	0.16
		$n^b = 6$	$n = 6$

[a] The main component of 2,4-DNPH.
[b] The number of experiments.

exception of etiocholanolone and dehydroepiandrosterone. These two steroids are successfully separated in the systems containing chloroform–acetone (Fig. 1). In these systems, androsterone, dehydroepiandrosterone and etiocholanolone were separated particularly successfully.

The separation of 11-substitued isomers, which differ in the 5α-(androsterone) and 5β-(etiocholanolone) configuration, was carried out preferably in system E. An 11β-hydroxy and an 11-oxo group with rings A/B in the *cis* configuration is less polar in all the systems used here than the isomer with rings A/B in the *trans* configuration. An 11-oxo group is more polar than an 11-hydroxy group in system E and less polar



...dimensional separation of 2,4-dinitrophenylhydrazones of 17-ketosteroid... numbers ...are as noted in Table I.

in the system where chloroform is the principal component. This fact is of great value in the selection of systems for two-dimensional chromatography.

Two-dimensional chromatography. The systems described in the previous section can be readily combined in a two-dimensional method to separate all the above 2,4-dinitrophenylhydrazones of 17-KS. Fig. 2 shows this separation. The plate was developed by two runs in the same direction to the edge in system E and in the second direction in system C. The numbers of the compounds are identical with those noted in Table I.

Separation is complete and can be used in the same way on the 20 × 10 cm plate when the differences in concentrations of the single 2,4-dinitrophenylhydrazones are not high. The application of these results to the analysis of the 17-KS in urine will be reported later.

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