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Note

Separation of epimeric 3-hydroxyandrostanes and 3-hydroxyandrostenes by thin-layer chromatography on silica gel

A. SUNDE, P. STENSTAD and K. B. EIK-NES
Institute of Biophysics, University of Trondheim, N-7034 Trondheim-NTH (Norway)
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Most target cells for androgens can reduce testosterone at the C-5 position to 17β-hydroxyandrostan-3-one (dihydrotestosterone). Dihydrotestosterone is believed to be the active androgen at the cellular level, and it can further be metabolized to 5α-androstane-3α, 17β-diol or 5α-androstane-3β, 17β-diol by many mammalian cells. In some cellular systems, testosterone can also be converted into 4-androstene-3α, 17β-diol and/or 4-androstene-3β, 17β-diol[1]. When tested in bioassays, these 3,17-dihydroxy-C_{19} steroids display different androgenicities, but their relative potency depends on the bioassay system used. It is not clear whether the 3,17-dihydroxylated androstanes or androstenes exert their biological activity via metabolism to dihydrotestosterone or testosterone, respectively. Studies on the metabolism of these steroids *in vivo* and *in vitro* must be carried out in order to resolve this question.

Methods for the separation of epimeric 3-hydroxy-C_{19} steroids are based on partition chromatography on paper[1,2] and adsorption chromatography on thin layers[3]. We have devised silica gel thin-layer chromatographic systems that separate the epimeric 3-hydroxyandrogens.

EXPERIMENTAL

Materials

All reagents used were of analytical-reagent grade (Merck, Darmstadt, G.F.R.) 2,7-Dichlorofluorescein was obtained from Sigma (St. Louis, Mo., U.S.A.). All steroids were obtained from Steraloids (Pawling, N.J., U.S.A.), except 4-androstene-3α,17β-diol, which was obtained from Merck.

Thin-layer plates were purchased from Schleicher & Schüll (Dassel, G.F.R.) with the following specifications: pre-coated silica gel plates F-1500, LS 254 W, Order No. 355099, 20 × 20 cm, 250-μm layer. Schleicher & Schüll manufacture two types of silica gel F-1500; Only the type with the order number cited above will display the chromatographic properties described in this paper. This type of silica gel F-1500 is referred to as the "old type" by Schleicher & Schüll, and the other as the "new type".

Methods

One-dimensional chromatography on pre-coated silica gel plates was carried

out in saturated tanks. Steroid samples were spotted 2.5 cm from the lower edge of the plate and at least 2 cm from the lateral border. The chromatograms were developed repeatedly by the ascending technique with dichloromethane–ethyl acetate (9:1) as mobile phase. The solvent front was drawn 16 cm from the application line.

Detection of steroids

After each development, the plates were allowed to air-dry, then lightly sprayed with a 0.2% ethanolic solution of 2,7-dichlorofluorescein and viewed under ultraviolet light (366 nm). All of the steroids used were clearly visible as bright zones.

RESULTS AND DISCUSSION

Fig. 1 and Table I summarize the results obtained. System I will easily and reproducibly separate all biologically important 5α-androstane metabolites. Chromatography with different amounts of steroids indicated that up to *ca.* 30 μg of each steroid, applied on a 2-cm long application line, will give satisfactory separations. A load of more than 50 μg of each steroid gives poorer separations. To our knowledge, no other liquid chromatography systems have been published that can separate all of these 5α-reduced androgens. The separation of 3α-hydroxy-5α-androstan-17-one (androsterone) from 3β-hydroxy-5α-androstan-17-one (epiandrosterone) on silica gel F or 1,2-propanediol-impregnated cellulose has been described[4]. These systems do not, however, separate dihydrotestosterone from epiandrosterone.

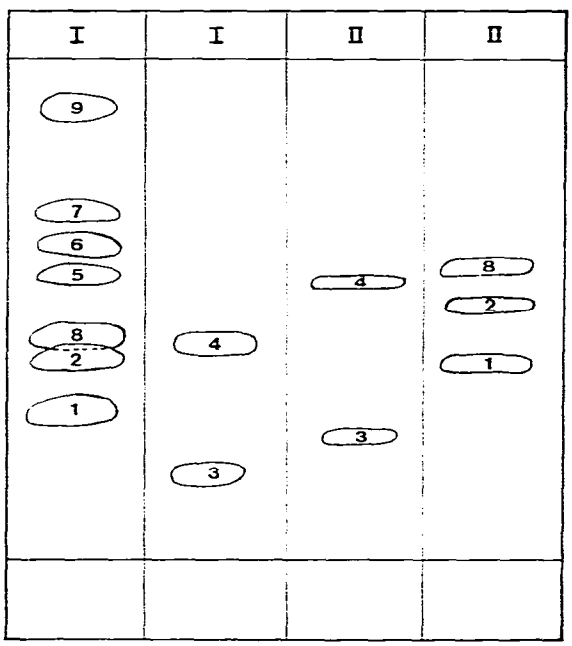

Fig. 1. Chromatography on silica gel F-1500 LS 254 W (Schleicher & Schüll, Order No. 355099) with dichloromethane–ethyl acetate (9:1). I = 2 developments; II = 5 developments at 4°. Numbers as in Table I.

TABLE I

R_s VALUES OF STEROIDS (RELATIVE TO 5α-ANDROSTANE-3α,17β-DIOL = 1.00) IN TLC WITH DICHLOROMETHANE–ETHYL ACETATE (9:1) AS MOBILE PHASE

No.	*Steroid*	R_s	
		*I**	*II**
1	5α-Androstane-3α,17β-diol	1.00	1.00
2	5α-Androstane-3β,17β-diol	1.30	1.27
3	4-Androstene-3α,17β-diol	0.57	0.45
4	4-Androstene-3β,17β-diol	1.45	1.42
5	3α-Hydroxy-5α-androstan-17-one	1.63	
6	3β-Hydroxy-5α-androstan-17-one	1.80	
7	17β-Hydroxy-5α-androstan-3-one	2.00	
8	17β-Hydroxy-4-androsten-3-one	1.48	1.50
9	5α-Androstane-3,17-dione	2.57	

* I = 2 developments; II = 5 developments at 4°.

The chromatographic separation of epimeric 3-hydroxy-4-androstenes has previously been achieved by partition chromatography of derivatives on paper[1]. This procedure is time consuming and has poor reproducibility. System II separates 4-androstene-3α,17β-diol from 4-androstene-3β,17β-diol. 5α-Androstane-3α,17β-diol and 5α-androstane-3β,17β-diol are also separated from each other and from the two androstenediols in this system.

The results given in Fig. 1 and Table I were obtained with the "old type" of Schleicher & Schüll silica gel F-1500. TLC with "new type" plates and our system I resulted in a good separation of 5α-androstane-3α,17β-diol from 5α-androstane-3β, 17β-diol. Dihydrotestosterone and androsterone were, however, not separated from each other on this type of silica gel F-1500 (data not shown). The chromatographic sequence on the two types of silica gel F-1500 is also different. The "old type" adsorbs 3α-hydroxyandrostanes more strongly than 3β-hydroxyandrostanes, while the "new type" adsorbs 3β-hydroxyandrostanes more strongly than 3α-hydroxyandrostanes. This chromatographic sequence is normally found on silica gel 60[3]. At present we do not know why the two types of silica gel F-1500 manufactured by Schleicher & Schüll display different chromatographic properties.

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