CHROM. 4196

A thin-layer chromatographic separation of some closely related 6-fluoro-16α-hydroxycorticosteroids

In recent years thin-layer chromatography (TLC) has become a useful tool in the separation[1-3] of closely related steroids and the resolution[4] of α- and β-steroid pairs. Several TLC systems for corticosteroids have been reported (*e.g.* refs. 5–14). It was found that these systems were not suitable for the adequate separation of a certain mixture of closely related 6-fluoro-16α-hydroxycorticosteroids that this laboratory was interested to separate and detect. The purpose of this paper is to describe a new solvent system for the separation of this mixture by TLC on silica gel using the one-dimensional multiple-development technique.

Experimental

Reagents. All solvents and chemicals were reagent grade.

Solvent system. The solvent system used was benzene–ethyl acetate (1:1).

Equipment. Pre-coated 250 μ thin-layer plates (Silica Gel F_{254}) were supplied by Brinkmann Instruments Inc., micro-pipets, Microcaps by Drummond Scientific Co., and Chromato-vue equipped with a short wavelength UV lamp (about 254 mμ) by Ultra-Violet Products Inc., Calif., U.S.A.

Procedure

The TLC plate was activated before use by heating at 110° for 1 h. The chromatography chamber was lined with filter paper dipped in the solvent system and allowed to equilibrate for 30 min before use to achieve complete saturation. The 6-fluoro-16α-hydroxycorticosteroid (Table I) solutions (1 mg/ml) made up in a mixture of chloroform–methanol (1:1) were spotted on the plate using a micro-pipet. Spots of 10 μg steroid were used. The plate was developed at room temperature, allowing the solvent front to move 15 cm from the point of application (about 45 min). The plate was then removed from the chamber and the solvent was allowed to evaporate for about 3 min at room temperature. The plate was then developed twice more in the same chamber as the first time. The solvent was completely dried at room temperature and the plate was viewed under the UV lamp to detect the separated spots. Measurements were made from the point of application to the center of the spot and the R_F value of each steroid was calculated according to GALLETTI[15] as the observed movement of the steroid after several runs (three in this system) divided by the distance of a single development (15 cm).

Results and discussion

Each of the 6-fluoro-16α-hydroxycorticosteroids listed in Table I was run singly and together with other members of the mixture. The average value for five determinations of the $\overline{R_F}$ value (× 100) for each of these steroids is given in Table I. The data show that the solvent system provides good separation of the steroid mixture used.

It was found that when the closely related 6α-fluoro-16α-hydroxy-11-desoxycortisone-16,17-acetonide was added to the mixture, its R_F (× 100) value was also 53, similar to that of 6α-fluoro-16α-hydroxycortisone-16,17-acetonide. Separation of the

TABLE I

R_F (× 100) VALUES FOR THE 6-FLUORO-16α-HYDROXYCORTICOSTEROIDS DEVELOPED IN BENZENE–ETHYL ACETATE (1:1)

Each figure represents the average of five independent determinations.

No.	*Steroid*[a]	R_F (× 100)
1	6α-Fluoro-16α-hydroxyhydrocortisone (flurandrenolone)[16]	5
2	6α-Fluoro-16α-hydroxyhydrocortisone-16,17-acetonide (flurandrenolone acetonide)[16]	25
3	6β-Fluoro-16α-hydroxyhydrocortisone-16,17-acetonide	36
4	6α-Fluoro-16α-hydroxyhydrocortisone-16,17-acetonide-11-acetate	45
5	6α-Fluoro-16α-hydroxycortisone-16,17-acetonide	53
6	6α-Fluoro-16α-hydroxyhydrocortisone-16,17-acetonide-21-acetate	61

[a] All steroids used in this work were obtained from Syntex Corporation, Palo Alto, Calif., U.S.A.

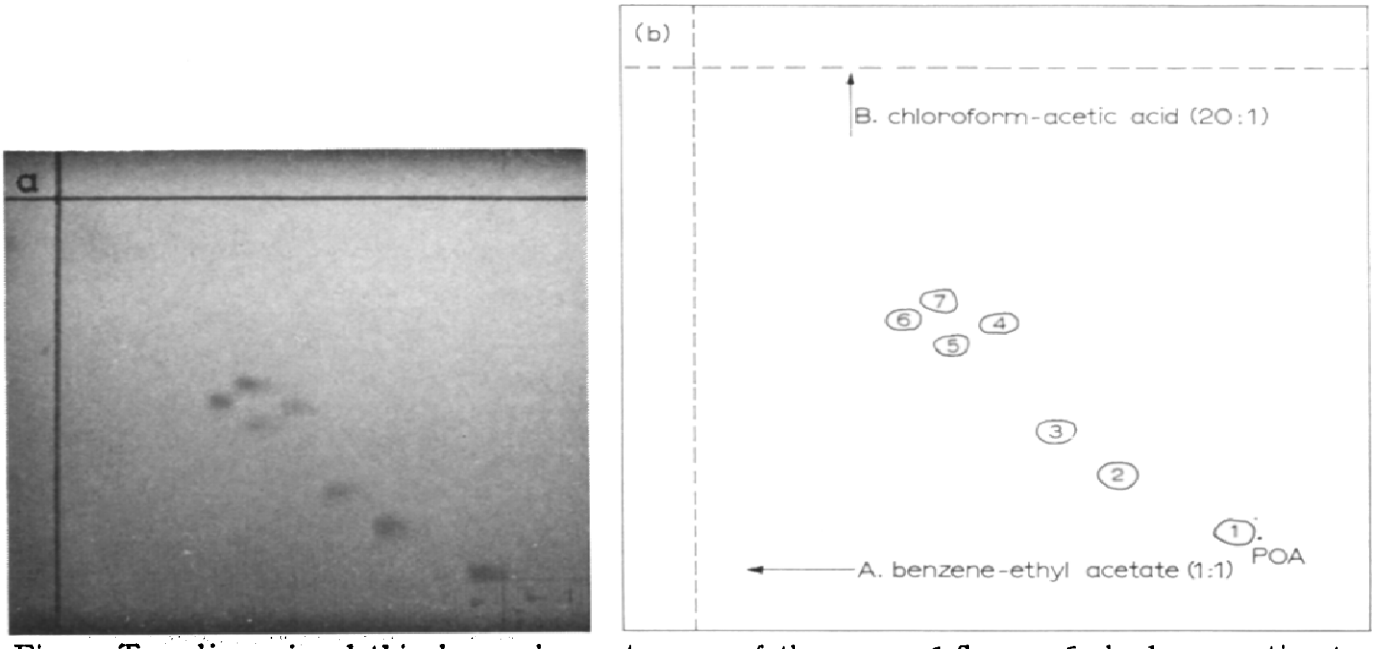


Fig. 1. Two-dimensional thin-layer chromatogram of the seven 6-fluoro-16α-hydroxycorticosteroids. The mixture spotted at the point of application (POA) contained 10 μg of each steroid. The plate was developed three times with benzene–ethyl acetate (1:1) in direction A. The chromatogram was then developed twice in direction B with chloroform–acetic acid (20:1). The numbers of the spots correspond to Table I. Spot No. 7 is 6α-fluoro-16α-hydroxy-11-desoxycortisone-16,17-acetonide. (a) Polaroid picture of the thin-layer chromatogram; (b) diagram of the thin-layer chromatogram.

two over-lying steroids was achieved by two-dimensional chromatography. Fig. 1 shows the separated mixture.

The solvent system reported in this paper has been used satisfactorily in this laboratory for the semi-quantitative determination of closely related foreign steroids that may be present in flurandrenolone acetonide. Upon the hydrolytic deacetonation[17] of the latter compound, flurandrenolone was separated by this solvent system. The

determination of the chromatographic purity of flurandrenolone acetonide by TLC using this system will be reported soon.

Analytical Development Laboratory, RAFIK H. BISHARA
Eli Lilly and Company, Indianapolis, Ind. (U.S.A.)

1 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 21 (1966) 488.
2 B. L. HAMMAN AND M. M. MARTIN, *Anal. Biochem.*, 20 (1967) 423.
3 R. H. BISHARA AND I. M. JAKOVLJEVIC, *J. Chromatog.*, 41 (1969) 136.
4 R. S. WRIGHT, *J. Chromatog.*, 37 (1968) 363.
5 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 9 (1962) 348.
6 O. ADAMEC, J. MATIS AND M. GALVANEK, *Steroids*, 1 (1963) 495.
7 O. NISHIKAZE, R. ABRAHAM AND H. STAUDINGER, *J. Biochem. (Tokyo)*, 54 (1963) 427.
8 P. AUDRIN, F. C. FOUSSARD, CH. BOURGOIN, L. JUNG AND P. MORAND, *Rev. Franc. Etudes Clin. Biol.*, 8 (1963) 507.
9 P. VECSEI, V. KEMENY AND A. GORGENYI, *J. Chromatog.*, 14 (1964) 506.
10 H. SUZUKI, *Nippon Naibumpi Gakkai Zasshi*, 40 (1965) 1358; *C.A.*, 63 (1965) 2071c.
11 W. HUBL, *Z. Chem.*, 6 (1966) 225.
12 A. UETTWILLER AND M. KELLER, *J. Chromatog.*, 35 (1968) 526.
13 N. H. CHOULIS, *Can. J. Pharm. Sci.*, 3 (1968) 76.
14 J. D. FEW AND T. J. FORWARD, *J. Chromatog.*, 36 (1968) 63.
15 F. GALLETTI, *2nd Trans. Meeting Study Group Steroid Hormones, Rome, 1965*, Publ. 1966, p. 189; *C. A.*, 68 (1968) 75444k.
16 *The Merck Index*, 8th Ed., Merck & Co., Inc., Rahway, N.J., U.S.A., 1968, p. 467.
17 G. R. ALLEN, JR AND M. J. WEISS, *J. Am. Chem. Soc.*, 82 (1960) 2840.

Received May 30th, 1969

J. Chromatog., 43 (1969) 539–541

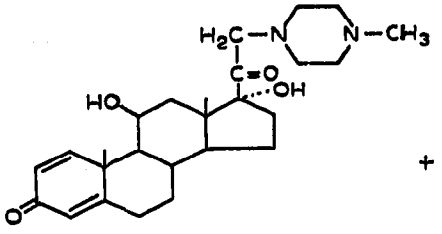
CHROM. 4200

Analysis of steroids

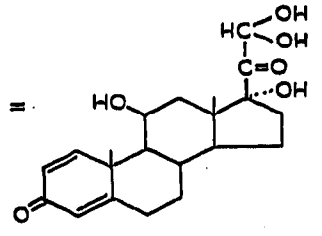
XIII. Detection of 21-amino corticosteroids on thin-layer chromatograms

During a study on the resorption and evacuation of 21-deoxy-21-N-(N'-methyl)-piperazinyl-prednisolone hydrochloride (Depersolone®) we needed a selective method for its detection on thin-layer plates. Tetrazolium methods can be applied for this purpose, but they cannot differentiate between common corticosteroids and their 21-amino derivatives.

The selective detection could be based on a recently reported reaction[1] which is highly specific for 21-amino corticosteroids:



$$+ 2\,HgCl_2 + CH_3COONa + 2\,H_2O =$$



$$+ Hg_2Cl_2 + NaCl + CH_3COOH +$$