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

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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.

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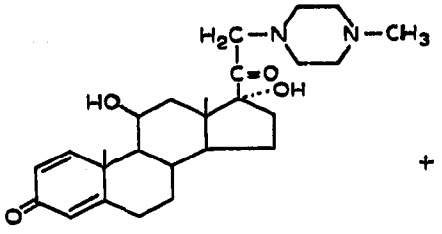
Analysis of steroids

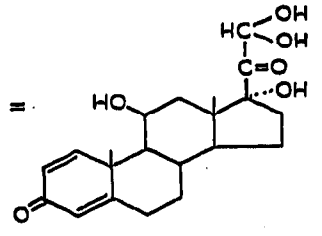
XIII. Detection of 21-amino corticosteroids on thin-layer chromatograms

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.

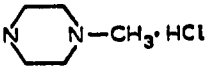
The selective detection could be based on a recently reported reaction[1] which is highly specific for 21-amino corticosteroids:



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



$$+ Hg_2Cl_2 + NaCl + CH_3COOH +$$



The plates were sprayed with methanolic solutions of mercuric chloride and sodium acetate followed by standing at room temperature in a chamber saturated with methanol vapour, in order to prevent evaporation of the solvent. During this period the reaction is nearly quantitative. (In the paper cited, a method is described for assaying Depersolone on the basis of the gravimetric determination of mercurous chloride formed in the reaction.) After evaporation of the methanol and spraying with ammonium hydroxide, the mercurous chloride turns to mercuric amido-chloride and metallic mercury and the latter can be observed as a grey spot.

Experimental

A mixture of 25 g Kieselgel G (Merck) and 50 ml water was applied to the glass plates at a thickness of 0.25 mm with a Desaga applicator. The plates were activated by heating for 1 h at 120°.

Peripheral bloodplasma, urine, liver and lung tissue were extracted with dichloromethane. These extracts were applied to the plates. Ascending development was carried out in a glass chamber equilibrated with the solvent. The chromatographic solvent was an acetone–methanol–triethanolamine (50:50:1.5) mixture. The length of run was 20 cm.

After evaporation of the solvent the plates were sprayed successively with 1 *M* methanolic solutions of sodium acetate and mercuric chloride. The plates were then immediately transferred into a chamber saturated with methanol vapour and laid in a horizontal position. After standing for 2 h, the layer was dried at room temperature and sprayed with a saturated aqueous solution of ammonia. The grey spot could instantly be observed in daylight.

Results

An R_F value of 0.42 was found for the free base of Depersolone. As little as 1 μg of Depersolone can be detected with the spray reagent described. Comparison of the spot density with reference spots permits a semiquantitative determination of Depersolone in a range of 1–100 μg.

The procedure described also proved to be suitable for the detection of other 21-amino corticosteroids, (*e.g.* substituted piperidyl and piperazinyl etc. derivatives).

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1 S. GÖRÖG, Z. TUBA AND I. EGYED, *Analyst*, 94 (1969) in press.

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