

CHROM. 3687

## Resolution of some pairs of closely related steroids by thin-layer chromatography

Work at present in progress in this laboratory has made necessary the development of TLC methods for the resolution of a number of pairs of closely related steroids

TABLE I

SEPARATION OF PAIRS OF CLOSELY RELATED STEROIDS ON STARCH-BOUND KIESELGEL N-HR/UV 254 (MACHEREY, NAGEL)

<i>Steroid pairs<sup>a</sup></i>	<i>No. of developments</i>	<i>Distance<sup>b</sup> moved (cm)</i>	<i>Separation factor</i>
<i>(a) Separations with cyclohexene-cyclohexanone (90:10)</i>			
5 $\alpha$ -Pregnane-3,20-dione	2	7.30	1.21
5 $\beta$ -Pregnane-3,20-dione		6.05	
5 $\alpha$ -Androstane-3,17-dione	2	5.40	1.23
5 $\beta$ -Androstane-3,17-dione		4.40	
3 $\beta$ -Hydroxy-5 $\alpha$ -pregnan-20-one	2	2.95	1.31
3 $\alpha$ -Hydroxy-5 $\beta$ -pregnan-20-one		2.25	
3 $\beta$ -Hydroxy-5 $\alpha$ -androstan-17-one	3	5.80	1.49
3 $\alpha$ -Hydroxy-5 $\beta$ -androstan-17-one		3.90	
<i>(b) Separations with cyclohexene-cyclohexanone (80:20)</i>			
3 $\beta$ -Hydroxy-5 $\alpha$ -androstan-17-one	1	6.50	1.18
3 $\alpha$ -Hydroxy-5 $\beta$ -androstan-17-one		5.50	
3 $\beta$ ,17 $\beta$ -Dihydroxy-5 $\alpha$ -androstan-17-one	2	3.35	1.43
3 $\alpha$ ,17 $\beta$ -Dihydroxy-5 $\beta$ -androstan-17-one		2.35	
3 $\beta$ ,20 $\beta$ -Dihydroxy-5 $\alpha$ -pregnane	2	4.40	1.33
3 $\alpha$ ,20 $\beta$ -Dihydroxy-5 $\beta$ -pregnane		3.30	
3 $\beta$ ,20 $\alpha$ -Dihydroxy-5 $\alpha$ -pregnane	2	3.00	1.36
3 $\alpha$ ,20 $\alpha$ -Dihydroxy-5 $\beta$ -pregnane		2.20	
3 $\beta$ ,17 $\alpha$ -Dihydroxy-5 $\alpha$ -pregnan-20-one	2	3.85	1.48
3 $\alpha$ ,17 $\alpha$ -Dihydroxy-5 $\beta$ -pregnan-20-one		2.60	
3 $\beta$ ,17 $\alpha$ ,20 $\beta$ -Trihydroxy-5 $\alpha$ -pregnane	3	2.50	1.47
3 $\alpha$ ,17 $\alpha$ ,20 $\beta$ -Trihydroxy-5 $\beta$ -pregnane		1.70	
3 $\beta$ ,17 $\alpha$ ,20 $\alpha$ -Trihydroxy-5 $\alpha$ -pregnane	3	1.80	1.71
3 $\alpha$ ,17 $\alpha$ ,20 $\alpha$ -Trihydroxy-5 $\beta$ -pregnane		1.05	

<sup>a</sup> Each pair was run as a mixture.

<sup>b</sup> Average of measurements on at least two chromatograms and quoted to nearest 0.5 mm. Solvents were freshly distilled. Plates were prepared by a method described earlier<sup>2</sup> and run in tanks lined with Whatman No. 3 papers. To avoid interference with detecting agents, last traces of cyclohexanone were removed from the plates by lightly spraying with water and drying in warm air. Many steroids were faintly visible while the plates remained damp<sup>3</sup>. The following detecting agents were employed: (1) 0.1% 2,4-dinitrophenylhydrazine in aqueous 2 N hydrochloric acid; (2) 10% phosphomolybdic acid in ethanol; (3) 0.002% aqueous rhodamine B, with examination under U.V. light of maximum output 355 m $\mu$  or, less satisfactorily, 254 m $\mu$ ; (4) sulphuric acid-water (1:1, w/v)<sup>5</sup>; this reagent produced some background darkening.

for example,  $5\alpha$ - and  $5\beta$ -androstande-3,17-dione,  $5\alpha$ - and  $5\beta$ -pregnane-3,20-dione and  $3\alpha$ -hydroxy- $5\beta$ - and  $3\beta$ -hydroxy- $5\alpha$ -pregnan-20-one<sup>1</sup>.

Preliminary experiments established that many of the pairs of closely related steroids could be resolved by repeated development on thin-layer plates in solvents of low polarity, for example, cyclohexane with up to 10% v/v of cyclohexanone, acetone or ethyl acetate. Solvents whose polarities had been increased by increasing the proportions of the more polar constituents showed reduced resolving power and were less satisfactory. Where solvents of increased polarity were required it was found advantageous to substitute cyclohexene for cyclohexane. As shown in Table I, solvent mixtures of cyclohexene and cyclohexanone have been used to resolve a number of pairs of closely related steroids of interest in this laboratory. Mixtures of cyclohexene with acetone or ethyl acetate have also been used. Although the disadvantages which accompany use of the high boiling cyclohexanone are thereby avoided, the resolving power of these solvents with some steroid mixtures was inferior to that of the cyclohexene-cyclohexanone systems.

#### *Acknowledgements*

This work forms part of the programme of the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland. Gifts of steroid samples from the Medical Research Council Steroid Reference Collection are acknowledged.

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First received May 30th, 1968; modified July 8th, 1968

*J. Chromatog.*, 37 (1968) 363-364

CHROM. 3702

### **Un nouveau mode de détection, sur couches minces, des amides substituées**

Les amides aliphatiques substituées peuvent être détectées par pulvérisation, soit de sulfate acide d'ammoniaque (suivie de chauffage), soit d'une solution d'iode, soit de techniques plus particulières dans le cas spécial des urées<sup>1</sup>.

La méthode que nous proposons consiste dans la pulvérisation successive d'une solution de dinitrophénylhydrazine (DNPH) et d'une solution d'iode.

L'action préalable du DNPH augmente très nettement les limites de détection de l'iode utilisé seul (cf. Tableau I) et, de plus, rend ce processus spécifique. En effet, sur le fond jaune obtenu par la pulvérisation du DNPH l'iode ne met pratiquement

*J. Chromatog.*, 37 (1968) 364-365