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CHROMATOGRAPHY OF STEROIDS ON A THIN LAYER OF CHARCOAL*

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In the contribution[1] at the Symposium at Frascati, it was attempted to show how we had succeeded, at least partly, in solving the difficult problem of the detection of colourless substances on a completely black support, *i.e.*, background. Fresh, wet charcoal chromatograms were copied by pressing on them filter-paper, which was then sprayed with detection reagents. We have now found that with steroids, copying by pressing a Silufol plate on the charcoal chromatogram is easier and more satisfactory, especially for UV-absorbing spots. However, after this treatment, Silufol does not remain completely white but usually becomes smeared with charcoal. If this greying of Silufol is not uniform, it is sometimes difficult to identify the true spot of the UV-absorbing substance that has been copied on to the Silufol. We also observed that substances with very low R_F values are not copied satisfactorily on to Silufol. We therefore tried another method of detection that was proposed in our earlier contribution[1], *i.e.*, scanning of the chromatograms after having chromatographed labelled steroids. Fig. 1 illustrates our experiments.

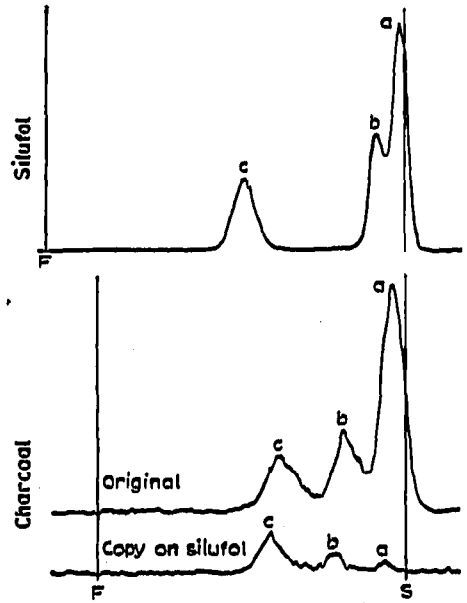

Fig. 1. Chromatograms of a mixture of labelled steroids on Silufol and charcoal thin-layer plates, and of the copy of the charcoal chromatogram on Silufol. S = start; F = front; a = [4-^{14}C]-estrone; b = [4-^{14}C]cholesterol; c = [4-^{14}C]cholesteryl acetate. Solvent, chloroform. Apparatus, Berthold-Frieseke Dünnschichtscanner II.

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Fig. 1 shows the separation of a mixture of steroids, *viz*, estrone, cholesterol and cholesteryl acetate. From the record of the radioactivity, it is evident that on Silufol the R_F values of estrone and free cholesterol are lower than on charcoal, while the opposite is true for cholesteryl acetate. This demonstrates the applicability of charcoal for chromatographic separations. In the lower part of Fig. 1, it can be seen that the scanning of a charcoal chromatogram of labelled steroids might also be used for quantitative purposes. However, the method of copying charcoal chromatograms is quite unsuitable for quantitative purposes. It can be predicted on theoretical grounds that substances with high R_F values will pass to a greater extent into the Silufol plate during copying, while substances with low R_F values will remain predominantly adsorbed on the adsorbent, in our case charcoal, and only a very small fraction will pass into the Silufol layer. Therefore, the heights of the peaks on the copy decrease with decreasing distribution ratio between the solvent, in our case chloroform, and the adsorbent. As the procedure of copying is not of a quantitative character (regular and reproducible), this dependence cannot be used to account quantitatively for the total radioactivity of the individual compounds.

REFERENCE

1 Ž. PROCHÁZKA, *J. Chromatogr.*, 48 (1970) 113.