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Thin-layer chromatography of DANSYL-oestrogens

In a previous communication[1], we described the use of derivative formation with 1-dimethylaminonaphthalene-5-sulphonyl chloride (DANSYL-chloride) as a means of determining oestriol in pregnancy urine. On viewing the fluorescent spots on a thin-layer plate, following chromatography of the DANSYL-oestrogens, one can observe any other oestrogen excreted in a significant amount, and we therefore considered it of value to record the behaviour of DANSYL-oestrogens on chromatography.

Table I shows the R_F values of 18 naturally-occurring oestrogens chromatographed both in the free state and as their DANSYL-derivatives.

TABLE I

R_F VALUES OF OESTROGENS CHROMATOGRAPHED ON KIESELGEL G PLATES, BOTH IN THE FREE STATE AND AS THEIR DANSYL-DERIVATIVES

The solvent was ethanol–chloroform (5:95).

Oestrogen	*R_F values*	
	Free compound	*DANSYL-derivative*
Oestrone	0.74	0.97
16-Keto-oestrone	0.59	0.12[a]
2-Methoxyoestradiol	0.53	0.79
16α-Hydroxyoestrone	0.46	0.83
16-Keto-oestradiol	0.46	0.83
2-Hydroxyoestrone	0.42	0.97
Oestradiol	0.38	0.83
11β-Hydroxyoestrone	0.29	0.80
2-Hydroxyoestradiol	0.25	0.83
17-Epioestriol	0.24	0.33
16-Epioestriol	0.24	0.32
2-Methoxyoestriol	0.15	0.25
6α-Hydroxyoestradiol	0.15	0.57
Oestriol	0.09	0.20
16,17-Epioestriol	0.09	0.21
6-Keto-oestriol	0.06	0.16
2-Hydroxyoestriol	0.045	0.25
6α-Hydroxyoestriol	0.03	0.14

[a] This is probably degraded. See comment in text.

In general, the only effect of the derivative formation is to raise the R_F value. An even higher elevation of R_F value is seen in the case of 2-hydroxy-oestrogens, which is presumably due to DANSYL-substitution on the phenolic 2-OH group as well as on the 3-OH. Thus, the retardation caused by the introduction of an extra hydroxyl group, seen among the free steroids, is not observed in the case of DANSYL-2-hydroxy-oestrogens.

Anomalous behaviour is also observed in the DANSYL-derivative of 16-keto-oestrone. This derivative would be expected to appear on the chromatogram with an R_F value slightly less than that of oestrone. Even if the derivative were to exist in the enolic form following the alkaline treatment, it should still have a moderately

high R_F value. Since, however, a spot is obtained with an R_F value of 0.12, it seems most probable that ring D undergoes fission under the conditions required for DANSYL-ation. It may, perhaps, be worth mentioning that 16-keto-oestrone, in common with 16-keto-oestradiol, may be chromatographed as DANSYL-16-epioestriol after reduction with borohydride.

In assessing the presence of unusual metabolites in pregnancy urine, it should be borne in mind that the DANSYL-ation reaction is not specific for oestrogens, but will occur with any phenolic OH group. This is not usually a serious drawback, and in more than 1000 oestriol determinations we have only twice been puzzled by the appearance of an unusual spot, with an R_F value similar to 16-epioestriol, in two patients receiving anticonvulsant drug therapy (diphenylhydantoin and luminal). That this spot was due to the drugs and not to the pregnancy was demonstrated when the same spot was obtained from a non-pregnant woman receiving similar treatment.

In pregnancy urine there is, however, a spot with an R_F value approximately half that of oestriol, that occurs to a minor extent in all pregnancies, but may reach high levels in some pathological conditions.

As we have mentioned previously[1], DANSYL-ation of oestrogens is applicable to pregnancy urines where oestrogen excretion is in excess of 1 mg per day. In non-pregnancy urines, the level of other phenolic material is comparable with, or in excess of, the amount of oestrogens, thus rendering differentiation difficult.

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