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SHORT COMMUNICATION

PREPARATION AND SOME PROPERTIES OF THE DANSYL ESTERS OF OESTRONE, OESTRADIOL AND OESTRIOL

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SUMMARY

The dansyl esters of oestrone, oestradiol and oestriol were prepared in crystalline forms and were characterized by their elemental analyses and some of their spectroscopic and chromatographic properties.

WELLS[1] introduced a method for the fluorimetric determination of oestrogens based on the initial formation of their 1-(N,N-dimethylamino)-naphthalene-5-sulphonates (dansyl esters). The author briefly referred to the crystalline dansyl ester of oestradiol but did not report the experimental details of its preparation. The recent interest in the analytical method of Wells[2–5] prompts us to report the preparation and some of the properties of the crystalline dansyl esters of oestrone, oestradiol and oestriol.

General operations. Ultraviolet spectra were taken with the Unicam SP8000 recording spectrophotometer, fluorescence spectra with the Aminco–Bowman spectrophotofluorimeter. Samples for analysis were dried at 90°C and 0·1 mm Hg for at least 3 h. M.p.'s were determined on a Kofler stage and were not corrected. T.l.c. was performed on 0·2 mm-thick layers of silica gel (Camag, DS-5). Acetone was refluxed over $KMnO_4$ and then distilled.

Oestrone dansyl ester. Oestrone (200 mg) in aqueous acetone (75%, v/v; 120 ml), dansyl chloride (600 mg) and M-$NaHCO_3$ (10 ml) were stirred 4 h at room temperature and the mixture was then left to stand overnight. Water (10 ml) and glycine (300 mg) were added and the mixture stirred for 4 h at room temperature. Acetone was then evaporated *in vacuo* at 30°C and the remaining aqueous phase was diluted with M-NaOH (50 ml) and then extracted with *n*-hexane (2 × 100 ml). The extracts were washed with water (2 × 50 ml), combined and, without removing some suspended solid material, taken to dryness. The crude product (335 mg; 90% of theory) gave from acetone-*n*-hexane brilliant yellow crystals of m.p. 185–186°C, λ_{max}^{EtOH} 216, 256 and 347 nm (ϵ 50,000, 14,500 and 3,900 respectively). (Found: C, 71·4%; H, 6·4%; N, 2·6%; S, 6·6%. $C_{30}H_{33}O_4NS$ requires: C, 71·5%; H, 6·6%; N, 2·8%; S, 6·4%.

Oestradiol dansyl ester. Oestradiol (120 mg) in aqueous acetone (60%, v/v; 50 ml), dansyl chloride (250 mg) and M-$NaHCO_3$ (4 ml) were allowed to react essentially under the conditions outlined above. Glycine (200 mg) was then added and the mixture stirred for 4 h at room temperature. After evaporation of the acetone the precipitated crystalline product was filtered off, washed with water and dried *in vacuo* over silica gel. The yield was 99%, the m.p. 183–185°C and after recrystallisation from acetone–*n*-hexane 184–185°C, λ_{max}^{EtOH} 216, 256 and 347 nm (ϵ 52,000, 14,500 and 3,800 respectively). (Found: C, 71·5%; H, 7·0%; N, 2·7%; S, 6·4%. $C_{30}H_{35}O_4NS$ requires: C, 71·3%; H, 6·9%; N, 2·8%; S, 6·3%).

Oestriol dansyl ester. Oestriol (150 mg) in aqueous acetone (60%, v/v; 35 ml),

dansyl chloride (400 mg) and M-$NaHCO_3$ (3 ml) were allowed to react and the reaction mixture treated essentially as in the preparation of the oestrone derivative except that dichloromethane was used for extraction. The crude product was obtained in theoretical yield. From acetone-*n*-hexane it gave crystals of m.p. 220–221°C, λ_{max}^{EtOH} 216, 256 and 347 nm (ϵ 50,000, 14,200 and 3,700 respectively). (Found: C, 69·0%; H, 6·7%; N, 3·0%; S, 6·1%. $C_{30}H_{35}O_5NS$ requires: C, 69·1%; H, 6·8%; N, 2·7%; S, 6·2%.)

Isolation of pure products was facilitated by reacting surplus dansyl chloride with glycine. Owing to their solubility in water the formed N-dansylglycine and surplus glycine were removed by the precipitation of oestradiol dansyl ester on evaporation of acetone. In the two other preparations, the separation was brought about by partition between an organic solvent and alkali. The latter procedure was found to be useful also in the microanalytical application of the reaction.

Table 1. T.l.c. of oestrogen dansyl esters

Dansyl ester of	T.l.c. system A*		B†	
	R_F	ΔR_M‡	R_F	ΔR_M‡
Oestrone	0·82	−0·76	0·40	0·21
Oestradiol	0·51	−0·68	0·37	0·23
Oestriol	0·07	−0·77	0·13	0·23

*Methanol, chloroform (1 : 49, v/v).
†2-Methylpropan-2-ol, 2,2,4-trimethylpentane (3 : 7, v/v).
‡Relative to the parent compound.

All three dansyl esters exhibited identical fluorescence spectra with maximal intensity of fluorescence at 500 nm and maximal excitation at 360 nm. Their intensity of fluorescence in dioxan was 0·4 that of quinine in 0·05 M H_2SO_4 on a molar basis. At concentrations above 20 ng/ml there was a linear relationship between concentration and emission at 500 nm. Below this concentration the stray light from the excitation beam began to contribute significantly to the reading at 500 nm. The three dansyl esters were chromatographed on a thin layer of silica gel in two different solvent systems (A and B; see Table 1). It is of interest to note that, in the two systems used, esterification had opposite effects on mobility. Following chromatography in system A and exposure of the chromatogram to the vapour of dioxan [6, 7] 2 ng of a dansyl ester could be detected under U.V. light (250 nm). After chromatography in system B, the limit of detection was 5 ng.

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