LETTERS TO THE EDITOR

Effect of Hydrogen Peroxide on the Colour of the Fluorescence of Oestrone in Concentrated Sulphuric Acid

SIR,—Oestrogens in solution in concentrated sulphuric acid when irradiated with ultra-violet light have fluorescent intensities which differ sufficiently to allow the detection and differentiation of oestrone, oestradiol and oestriol from one another on paper chromatograms and for their quantitative assay (Ittrich, 1960; Preedy and Aitken, 1961). When dissolved in amounts of approximately 1 μg./ml. of sulphuric acid, oestrone fluoresces green-yellow; oestradiol, gold-yellow, and oestriol, orange-yellow.

The addition of small amounts of 30 per cent aqueous hydrogen peroxide to such solutions affects their ability to fluoresce: oestrone emits a blue fluorescence of high intensity, whereas with oestriol and oestradiol, the fluorescence is quenched.

The blue fluorescence of oestrone was used to determine its presence when in admixture with oestradiol and oestriol. A Pulfrich photometer with ultra-violet equipment was used with a C comparator plate as a standard light source.

From an ethanolic (95 per cent w/v) solution of oestrone (10 μg./ml.), appropriate amounts are transferred to Jena-glass test-tubes of inner diameter 8 mm. The ethanol is evaporated and each dry residue dissolved in 93·5 per cent sulphuric acid (1 ml.). This is followed immediately by the addition of 30 per cent hydrogen peroxide (0·05 ml.). Transference of the tubes to the photometer and measurement of the intensity of the blue fluorescence must be made after 5 min. and at a temperature of 22°. These are the optimal conditions for the determination. The intensity of the blue fluorescence increases for a short time after the addition of hydrogen peroxide and then decreases; it varies with the concentration of sulphuric acid used, the amount of peroxide added and the temperature. Hence, it is necessary to adhere strictly to the conditions of the procedure.

From a graph, plotting the intensity of the blue fluorescence against the amount of oestrone, a linear relation was found over the range of 10–40 μg. of the drug.

Oestrone can be determined quantitatively in this way even though the apparatus used has a relatively low sensitivity. The fluorescence of oestrone solutions in sulphuric acid without the addition of hydrogen peroxide is too weak to make this quantitative determination possible.

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May 8, 1963

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