

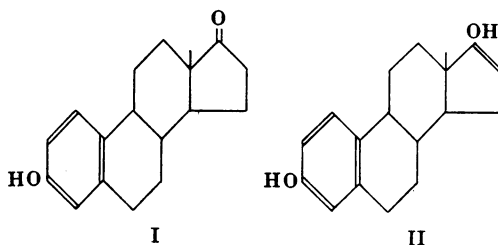
CXXXII. THE ABSORPTION SPECTRA OF OESTRONE AND RELATED COMPOUNDS IN ALKALINE SOLUTION.

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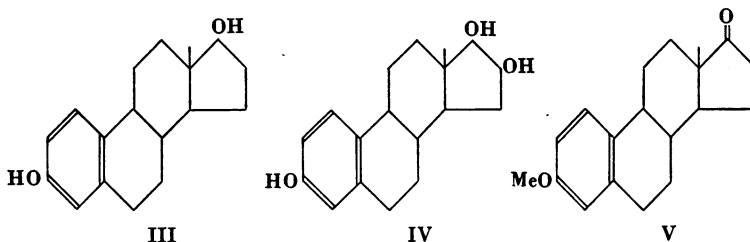
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PEDERSON-BJERGAARD AND SCHOU [1935] recently measured the absorption spectrum of oestrone in aqueous-alcoholic solution and found that the peak of the absorption band moved from about 2800 Å. in acid or neutral solutions to about 2950 Å. in alkaline solution, whilst the intensity also increased slightly. They attributed this change to enolisation of the 17-keto-group and the influence of the new ethenoid linkage produced, as shown in formulae I and II.



It seemed improbable that introduction of an isolated ethenoid linkage would produce this change in absorption spectrum, which might better be attributed to salt formation by the phenolic 3-hydroxy-group. Strictly analogous changes occur in the case of simple phenols when they form phenoxides, as has been shown by Baly and Ewbank [1905] and by Ley [1920].

The matter was, therefore, put to the test by measuring the absorption spectra, in neutral and alkaline aqueous-alcoholic solution, of two oestrin derivatives in which tautomerism in ring IV is impossible, but which can form phenoxides: namely oestradiol (III) and oestriol (IV). Oestrone methyl ether (V) in which enolisation is conceivable but salt formation is impossible was also investigated. The measurement of the absorption spectrum of oestrone was repeated under the same conditions.



As may be seen in the diagrams (Fig. 1), oestrone, oestradiol and oestriol all behave similarly. One molecular proportion of NaOH in the concentration used

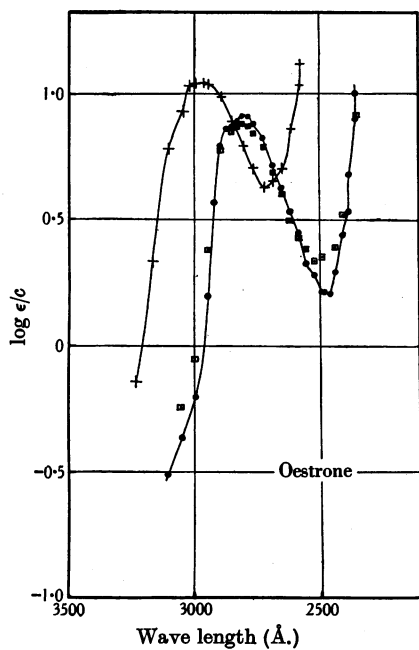


Fig. 1 a.

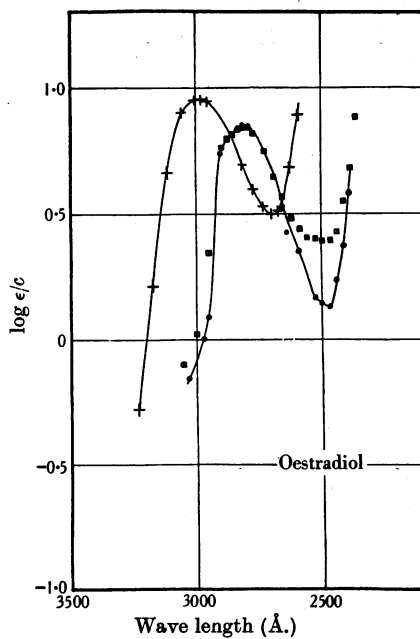


Fig. 1 b.

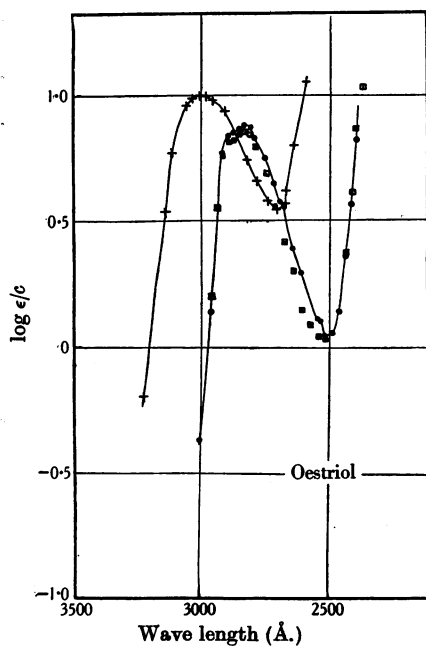


Fig. 1 c.

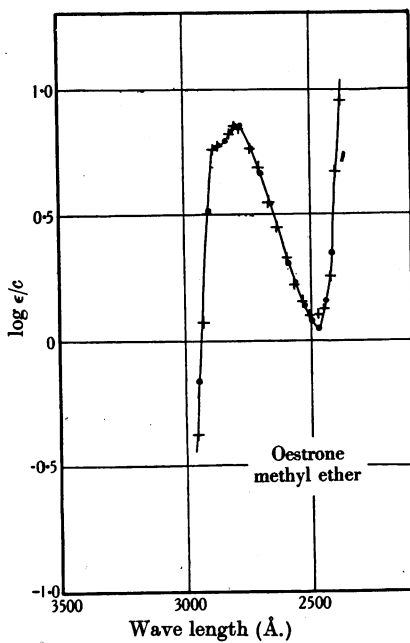


Fig. 1 d.

Fig. 1. Absorption spectra of oestrone (a), oestradiol (b), oestriol (c), and oestrone methyl ether (d) in 90% alcohol; neutral ●, *N*/3000 NaOH □, *N*/100 NaOH +.

(*ca.* $N/3000$) produced little effect, whilst 30 molecular proportions shifted the maxima to 2950–3000 Å. (Fig. 1, *a, b, c*). On the other hand, the absorption spectrum of oestrone methyl ether was not affected by an excess of NaOH (Fig. 1 *d*). It is, therefore, demonstrated that the change in absorption spectrum in alkaline solution is to be attributed to the formation of phenoxide. No evidence is afforded whether enolisation occurs or not. It may be pointed out that this method of examination is diagnostic of a free phenolic hydroxy-group, and should be applicable to natural esters of oestrone or oestriol (such as the oestriolglucuronic acid of Cohen and Marrian [1936]) in the same way that absorption spectra have been applied to the determination of the constitution of the purine nucleosides by Gulland *et al.* [1934].

EXPERIMENTAL.

Materials. The M.P. (uncorr.) of the substances used were as follows: (*a*) oestrone, 255–257° ($[\alpha]_D^{18}$, +165°); (*b*) oestradiol, 170–172°; (*c*) oestriol, 279–280°; (*d*) oestrone methyl ether, 167–169°: specimens (*a*) and (*c*) were given by Dr A. Girard to Dr O. Rosenheim, to whom I am indebted for passing them on, (*b*) was purchased from British Drug Houses, Ltd., (*d*) was prepared from oestrone as described by Cohen *et al.* [1935].

Measurements. The absorption spectra were measured by the method of Philpot and Schuster [1933], and are expressed as $\log \frac{\epsilon}{c}$ where

$$\epsilon = \frac{1}{d} \log_{10} \left(\frac{\text{incident}}{\text{transmitted}} \text{ light} \right) - \epsilon_0$$

and *c* is the concentration in g. per l.

The substances were made up in about 0.01 % solution in absolute alcohol. 5 ml. portions of this were diluted with (i) 0.5 ml. of water, (ii) 0.5 ml. of aqueous $N/300$ NaOH and (iii) 0.5 ml. of aqueous $N/10$ NaOH and photographed. Comparison spectrographs of the solvent were made from the same mixtures of alcohol with aqueous NaOH. The actual final concentrations used were: oestrone, 0.00969 %; oestradiol, 0.00960 %; oestriol, 0.00949 %; oestrone methyl ether, 0.00936 %.

SUMMARY.

Addition of sodium hydroxide to solutions of oestrone, oestradiol or oestriol causes a shift of the ultraviolet absorption band towards the visible region. The absorption of oestrone methyl ether is unaffected. The change in absorption is therefore due to salt formation by the phenolic group in the 3-position, and is not to be explained in the case of oestrone by enolisation of the 17-keto-group.

I am indebted to Mr E. H. Pitte for technical assistance in these measurements.

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