A Colour Reaction of Diethylstilbœstrol (4.4′ dihydroxy-α, β-diethylstilbene)

DODDS *et al.*[1] discovered a series of non-steroid œstrogenic compounds, 4.4′-dihydroxy-α, β-diethylstilbene, or diethylstilbœstrol, being the most potent representative of this series. In addition to its œstrogenic effect, this compound has other effects in common with the steroid type of œstrogens; for example, it opens the vagina of infantile rats and mice, it stimulates the evolution of the uterus and prevents the appearance of 'castrate'-cells in the ventral pituitary.

Besides these effects, others have been found, for which the natural and artificial œstrogens are no longer interchangeable.

Mühlbock[2] reported on the inhibition of comb growth by the simultaneous administration of male substances with œstrogens. The natural œstrogens act in this way when administered subcutaneously or by inunction; diethylstilbœstrol, however, is active only when administered subcutaneously.

Loeser[3] claims that diethylstilbœstrol, in contradistinction to natural œstrogens, has no influence on the thyroid after intrauterine application. Levie[4] and Gaarenstroom and Levie[5] found that stilbœstrol hastens the involution of epiphyseal cartilage more than œstrone. Duyvené de Wit[6] observed no lengthening of the ovipositor of *Rhodeus amarus* by stilbœstrol.

These effects may be suitable for discriminating biologically between a solution of natural from one of artificial œstrogens, but are unlikely to provide reliable quantitative information on mixtures of the two substances. The exact estimation of small quantities of diethylstilbœstrol in solutions of œstrone or œstradiol has now been achieved, however, by means of a colour reaction.

Addition of a few drops of a 50 per cent solution of antimony pentachloride to a solution of a few micrograms of stilbœstrol in chloroform produces a fuchsin-red colour, while in more concentrated solutions a bordeaux-red precipitate is obtained. By warming the mixture the reaction becomes more sensitive; 1 γ of stilbœstrol per c.c. of chloroform can still be detected, which amounts to a dilution of 10^{-6}. The colour attains its maximum intensity after 15 min. and remains constant for another 10–15 min.

For the detection of stilbœstrol in an oily solution of natural œstrogens the fatty and unsaponifiable substances must be removed prior to the colorimetric assay, because these substances would produce a dark brown discoloration. At a thousand times higher concentration, namely, 1 mgm./c.c., natural œstrogenic substances (steroids) produce a yellow-brown coloration with antimony pentachloride. The removal of these steroids from the solution will be described elsewhere.

It is important to use alcohol-free chloroform as a solvent, since in the presence of alcohol the red colour rapidly changes to blue-violet. For the colorimetric estimation a standard diethylstilbœstrol solution of similar strength to the unknown is used. The colorimetric assay has been successfully applied to the estimation of diethylstilbœstrol in the urine and the liver of dogs after subcutaneous injection of the hormone. The amounts found colorimetrically were the same as those ascertained by biological assay. The urine extracts were purified before carrying out the assay.

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[1] Dodds, E. C., Golberg, Lawson and Robinson, NATURE, **142**, 34 (1938).
[2] Mühlbock, O., NATURE, **143**, 160 (1939).
[3] Loeser, A., *Z. exp. Med.*, **105**, 430 (1939).
[4] Levie, L. H., Thesis, Amsterdam (1939).
[5] Gaarenstroom, J. H., and Levie, L. H., *J. Endocrin.*, **1**, No. 4 (1939).
[6] Duyvené de Wit J. J., *Klin. Wochenschr.*, **18**, 1423 (1939).

Points from Foregoing Letters

E. J. Williams and G. R. Evans submit photographs providing further evidence for the transformation of mesotrons into electrons, the first evidence for which was obtained at the same laboratory a short time ago. The experiments were carried out in a high-pressure cloud chamber containing argon at a pressure of 80 atmospheres.

H. J. Bhabha has extended his classical theory of mesons to include a treatment of the dipole moment, and shows that all the infinities can be removed. The cross-section for the scattering of neutral mesons by neutrons calculated from this theory agrees with the quantal result for small energies, but is much less than the latter for high energies, decreasing as the square of the energy. The result shows that the theory does not lead to Heisenberg explosions when proper account is taken of the damping forces.

In a note on the nature of oxide films on alloy steels, A. G. Quarrell criticizes the reasoning on which T. Tokumitu based his conclusion that the natural oxide film formed on stainless steel is α-$(Fe,Cr)_2O_3$. Recent application of the high temperature electron diffraction technique leads to the suggestion that the oxidation resistance of heat-resisting steels may be due to the formation of a stable spinel oxide which undergoes no lattice changes on heating and cooling.

F. G. Baddar finds that *o*-α-naphthylbenzoic acid and *o*-4′-methyl-1′-naphthylbenzoic acid give on ring closure a mixture of a benzanthrone and a benzfluorenone. Interaction between diazotized methyl anthranilate and β-methylnaphthalene gives *o*-2′-methyl-1′-naphthylbenzoic acid.

R. Pulver and F. Verzár, in continuation of their experiments on the role of potassium in carbohydrate metabolism, show that in yeast cells intake of potassium is simultaneous with glycogen formation, and that with the breakdown of glycogen potassium is again liberated. This agrees with their previous findings on the role of potassium in carbohydrate metabolism in animals.