

RESEARCH PAPERS

THE IDENTIFICATION OF THE SYNTHETIC STILBENE ŒSTROGENS

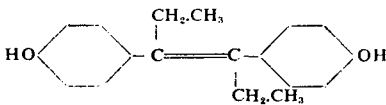
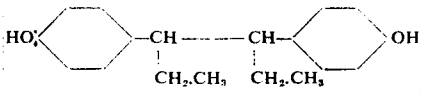
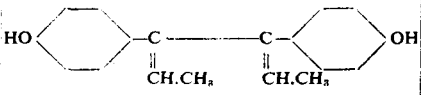
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FOLLOWING the demonstration by Dodds¹ of the œstrogenic activity of certain synthetic stilbene derivatives, various compounds of this type have been introduced into œstrogen therapy. Of these, the structurally similar stilbœstrol, hexœstrol and dienœstrol, and the dipropionate of stilbœstrol (Table I) are commercially available in this country, either in tablet form, in oil solution for injection purposes, or as ointments.

TABLE I
SYNTHETIC STILBENE ŒSTROGENS

| | Structure | M.pt. C. | Solubilities |
|---------------------------------|--|-------------|--|
| Stilbœstrol B.P. |  | 169 | Very slightly soluble water Slightly soluble benzene Soluble ethyl alcohol, ether, acetone, chloroform, sodium hydroxide |
| Hexœstrol B.P. |  | 186 | Insoluble water Slightly soluble chloroform, benzene Soluble ethyl alcohol, ether, acetone, sodium hydroxide |
| Dienœstrol B.P. |  | 233 | Insoluble water Slightly soluble chloroform, benzene Soluble ethyl alcohol, ether, acetone, sodium hydroxide |
| Stilbœstrol dipropionate B.P.C. | | 105 | Slightly soluble water Soluble ethyl alcohol, ether, acetone, chloroform, benzene |

The toxicity of these drugs is of the same order as that of the natural œstrogens, and toxic symptoms during clinical usage may develop as a result of excessive doses or prolonged administration. Although various colorimetric procedures are available for the quantitative determination of these synthetic products (Dingemans²; Tubis and Bloom³; Huf and Widmann⁴; Malpress⁵), their qualitative identification and differentiation appear to have received but little attention. The interesting colour reactions of Cocking^{6,7} whilst clearly differentiating hexœstrol from stilbœstrol and dienœstrol, permit of no distinction between the two latter compounds themselves, and are entirely negative in the case of the ester.

In the present publication a series of five simple colour reactions is described, from the results of which the three parent œstrogens and stilbœstrol dipropionate may be readily identified and differentiated.

SYNTHETIC STILBENE ŒSTROGENS

EXPERIMENTAL

In various instances the tests may be performed directly on tablets, oil solutions or ointments with entirely satisfactory results, but, in general, it is desirable for the drug to be isolated in the pure state, prior to the examination.

Colour reactions. The complete series of five tests described below requires, for any particular compound, a quantity of material of not more than 0.5 mg.; the resulting colours (Table II) are distinctive and free from ambiguity.

TABLE II
COLOUR REACTIONS OF SYNTHETIC STILBENE ŒSTROGENS

| Test | Stilbœstrol | Hexœstrol | Dienœstrol | Stilbœstrol Dipropionate |
|--------------------------------|-------------------------------|---|-----------------------------------|--|
| Sulphuric acid | Bright red yellow | Colourless | Yellow | Orange-yellow |
| Heated | Orange | Colourless | Green | Orange |
| Sulphuric acid-sodium nitrite | Intense red | Intense purple | Intense red | Intense red |
| Sulphuric acid-vanillin ... | Orange-red ; purple margin | Yellow | Greenish-brown ; purple margin | Orange-reddish- brown ; purple streaks |
| Addition of ethyl alcohol ... | Violet | Colourless | Pink | Violet |
| Phosphoric acid-vanillin ... | Yellow-purple- carminé | Yellow-reddish- brown-purple; solution turbid | Yellow-deep blue | Yellow-purple |
| Hydrochloric acid-vanillin ... | Colourless | Colourless | Blue | Colourless |
| Addition of bleaching powder | Green or blue | Colourless | — | Green |

(1) *Sulphuric acid test.* A quantity of material of the order of 0.01 to 0.1 mg. in a white porcelain dish is treated with one drop of concentrated sulphuric acid, and heated for 1 minute on a boiling water-bath.

(2) *Sulphuric acid-sodium nitrite test.* 0.1 mg. of sodium nitrite is treated with one drop of concentrated sulphuric acid in a white porcelain dish, which is then heated for 10 seconds on a boiling water-bath. 0.01 mg. of the test material is sprinkled over the surface of the still hot acid mixture.

(3) *Sulphuric acid-vanillin test.* 0.1 mg. each of test material and vanillin is placed in a white porcelain dish, and treated with one drop of concentrated sulphuric acid. The dish is warmed for one minute on a boiling water-bath with occasional gentle rocking and cooled, and the acid mixture is treated with 1 to 2 ml. of alcohol.

(4) *Phosphoric acid-vanillin test.* 0.01 to 0.1 mg. each of test material and vanillin is treated in a small (2" × ¼") test tube with one drop of syrupy phosphoric acid, and the liquid heated to boiling for 10 to 15 seconds.

(5) *Hydrochloric acid-vanillin test.* 0.1 mg. each of test material and vanillin in a 3" × $\frac{3}{8}$ " test tube is treated with one drop of glacial acetic acid, solution being affected by gentle warming. 2 to 3 drops of concentrated hydrochloric acid are added, and the liquid gently boiled for 10 to 15 seconds. If no colour develops, approximately 0.1 mg. of bleaching powder is added, and the liquid again boiled.

Although the results of this series of tests provide conclusive identification within the group, the conclusion should be checked whenever practicable by a micro mixed melting-point determination.

SUMMARY

1. A series of five simple colour reactions is described whereby the synthetic stilbene œstrogens, stilbœstrol, hexœstrol, dienœstrol and stilbœstrol dipropionate may be identified and distinguished.

2. The tests involve the use of no unusual reagents and may be completed within 5 to 10 minutes, using a total amount of only 0.5 mg. of test material.

The authors wish to express their thanks to Messrs. Boots Pure Drug Co. for their kind co-operation in supplying samples of pure œstrogens, and to Dr. H. S. Holden, Director of the Metropolitan Police Laboratory, for his permission to publish the results of this investigation.

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