LETTERS TO THE EDITOR

Colour and Fluorescence Reactions for Steroid and Synthetic Hormones.

FOR some time it has been known that warming with concentrated sulphuric acid caused condensation of several natural œstrogens with the production of coloured solutions with varying fluorescence effects. Boscott[1] has recently developed the phosphoric acid reaction of Finkelstein, Hestrin, and Koch[2] for the detection and estimation of steroid and synthetic œstrogens. The need for a reaction to distinguish between tablets containing small amounts of the various steroid and synthetic hormones in general has led us to investigate the possibility of extending Boscott's technique for this purpose, since it is known that the presence of small amounts of tablet disintegrants and lubricants interferes with certain colour reactions for these substances (cf. Cocking[3]). The basic technique described by Boscott was followed after preliminary extractions (when necessary) of the crushed tablets with ether and evaporation of the solvent. It is not certain in cases where extraction had to be used what accompanying tablet constituent was interfering with the fluorescence reaction; starch interfered with the dienœstrol reaction but in other cases merely caused a slight alteration of the fluorescence colour. The results given by a number of steroid hormones not previously examined are also recorded.

STEROID HORMONES.

The crystalline hormone was dissolved in 0·2 ml. of glacial acetic acid, mixed with about 2 ml. of 88 per cent. phosphoric acid and allowed to stand for 1 hour, the colour and fluorescence (under filtered ultra-violet light) being observed at intervals. After 1 hour the solution was diluted with about 3 ml. of glacial acetic acid and the colour and fluorescence again noted. In addition, approximate fluorescence intensities are reported relative to œstrone as standard.

Desoxycorticosterone acetate. 1 mg. gave a violet fluorescence, weaker than œstrone after 1 hour but stronger on dilution (*ca.* 2 x œstrone).

Ethinyl œstradiol. 0·1 mg. gave an intense orange fluorescence. Intensity: 50 to 100 x œstrone. Two tablets (each 0·05 mg.) crushed and extracted with ether gave a similar reaction when applied to the evaporated ether extract.

Ethisterone (*ethinyl testosterone*). The reaction produced a dichroic (green-violet) solution in acetic and phosphoric acids, turning to deep red in 1 hour. Dilution with acetic acid gave a red solution with an intense peach coloured fluorescence (50 to 100 x œstrone). Tablets (5 mg.) gave this reaction without extraction.

Methyl testosterone. 1 mg. gave a strong yellow fluorescence (*ca.* 10 x œstrone). Tablets gave the same result without extraction using approximately one-fifth of one tablet (5 mg.)

Œstradiol. An immediate light green fluorescence stronger than œstrone and unchanged after 1 hour was produced by 0·1 to 1 mg. Dilution with acetic acid caused partial quenching.

Œstradiol dipropionate. 0·1 to 1 mg. gave no reaction in the cold; heating at 100°C. for 5 minutes initiated a reaction similar to that of œstradiol.

Œstradiol monobenzoate. 0·1 to 1 mg. gave no reaction in the cold but behaved similarly to the dipropionate after heating at 100°C. for 10 minutes. The behaviour of the dipropionate and the monobenzoate indicated that

hydrolysis was occurring at 100°C. and also that an OH group in the 3-position is necessary for the production of fluorescence.

Œstrone. A green fluorescence was produced after 1 minute. After extraction with ether followed by evaporation of the ether extract the residue from tablets of œstrone gave the same reaction.

Progesterone. The reaction produced a weak blue fluorescence; 5 to 10 mg. quantities were required to make the test effective.

Testosterone propionate. This gave no reaction in the cold. On heating at 100°C. for 2 minutes and standing for 1 hour, dilution with acetic acid produced a fairly strong yellow fluorescence. Heating for 5 minutes following a similar procedure gave a red colour with an orange-yellow fluorescence, fairly strong but relatively weaker than œstrone.

Synthetic Hormones.

Dienœstrol. After dissolving 0·1 mg. in 0·2 ml. of glacial acetic acid, adding 1·8 ml. of 85 per cent. phosphoric acid, allowing to stand for 1 hour and then heating for 1 hour at 100°C. followed by dilution with 3 ml. of acetic acid, a purple colour with an intense but rather unstable violet fluorescence was produced, as described by Boscott. This reaction could be applied to dienœstrol extracted from crushed tablets with ether. Hexœstrol and stilbœstrol gave no reaction to this test.

With the exception of hexœstrol and stilbœstrol all the hormones mentioned can be distinguished when in tablet form by means of the colour and fluorescence reactions described, using, when necessary, ether extraction to separate the hormone from interfering tablet material. Owing to the low intensity of some of the fluorescence effects produced, comparison of an unknown fluorescence with fluorescences produced by known hormones when treated similarly should be used to obtain a reliable fluorescence identification.

Finkelstein, Hestrin and Koch postulated that the production of a fluorescence depended on the presence of a conjugated double bond system and on the position of polar groups. The present results have indicated the importance of a free OH group in the 3-position and have shown that a 17-ethinyl group confers intense fluorescence activity.

Preliminary work on the application of the techniques described to the identification and possible estimation of steroid and synthetic hormones in oily solutions for injection has revealed difficulties which necessitate further study.

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References.

1. Boscott, *Nature*, 1948, **162**, 577.
2. Finkelstein, Hestrin and Koch, *Proc. Soc. exp. Biol., N.Y.*, 1947, **64**, 64.
3. Cocking, *Analyst*, 1943, **68**, 144.

Correction.—No. 1, p. 61, line 2, for 500 read 550.