J. Pharm. Pharmac., 1967, **19**, 41–44 Received May 23, 1966

A note on spectrophotometric methods for the determination of norethynodrel and mestranol in tablets

R. A. BASTOW

The method for assay of norethynodrel involves acid catalysed rearrangement of the Δ5,10 system to a Δ4,3-ketone with stronger ultraviolet absorption. Mestranol is determined from its absorption at 280 mμ after elimination of the interfering ketonic absorption by reduction with borohydride. Residual interference is allowed for by a three point correction.

NORETHYNODREL (17-hydroxy-19-nor-17-pregn-5(10)-en-20-yn-3-one) and mestranol (17-hydroxy-3-methoxy-19-nor-17-pregna-1, 3, 5 (10)-trien-20-yne) occur together in a number of tablet formulations, and as the normal levels of mestranol are in the range 0·05 to 0·15 mg per tablet a sensitive analytical method is required. The widely used Kober reaction for oestrogens has undergone various modifications to improve precision and reproducibility. It is applicable to mestranol after preliminary separation from norethynodrel by chromatography but the technique proved to be tedious and of poor precision in this laboratory. Thin-layer and gas-liquid chromatography (Schulz, 1965) have also been proposed for the determination of mestranol in tablets. Legrand, Delaroff & Smolik (1958) determined oestradiol type steroids in the presence of large amounts of ketosteroids, such as testosterone or androstanolone, by ultraviolet spectrometry after reducton of the interfering ketone with potassium borohydride. Residual absorption by the reduction products was corrected graphically using data obtained on pure reduced samples of phenolic and ketosteroids.

This reduction is applicable to norethynodrel but pure samples of this drug, needed for obtaining data on the background absorption, are difficult to obtain because, as normally manufactured, it contains up to 1·5% of mestranol. Repeated recrystallization will not normally bring this below 0·5%. An alternative method of background correction is by means of a three point correction (Morton & Stubbs, 1946) and the method to be described uses this principle.

The technique of determining norethynodrel by direct ultraviolet absorption is unsatisfactory due to the lack of characteristic absorption and for this reason Chissell (1964) proposed a colorimetric method using *m*-dinitrobenzene. Norethynodrel under acid conditions rearranges to norethisterone (17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one) which has the characteristic conjugated ketone absorption at about 240 mμ suitable for its quantitative estimation.

Experimental

ACID CATALYSED REARRANGEMENT OF NORETHYNODREL

A solution of norethynodrel in methanol, 100 μg/ml, was prepared and 5 ml portions heated for different time intervals in a boiling water bath

From Pfizer Ltd., Sandwich, Kent.

with N hydrochloric acid (5 ml). The resultant solution was diluted with methanol (100 ml) and examined spectroscopically. The absorption at 241 mμ, brought about by rearrangement to norethisterone, was at a maximum E (1%, 1 cm) of 560 after 5 min heating and corresponded to complete isomerization.

DERIVATION OF BACKGROUND CORRECTION FOR MESTRANOL

A pure sample of mestranol was subjected to the borohydride reduction procedure described by Legrand & others (1958) and a three point correction calculated from a plot of the ultraviolet spectrum of the resultant solution (Fig. 1).

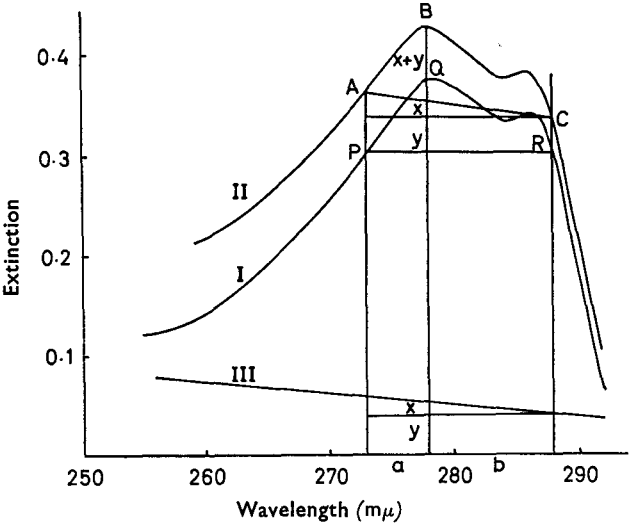

FIG. 1. Ultraviolet spectra of: I, mestranol; II, mestranol in the presence of background interference; III, background interference.

In the spectrum of pure mestranol, points P and R are of equal absorption and this absorption is a known fraction F of the absorption Q at the peak. By proportion in the similar triangles:

$$x = (A - C)\frac{b}{a + b}$$

and

$$\frac{C - y}{B - (x + y)} = \frac{R}{Q} = F$$

The required corrected absorption is $B - (x + y)$.

Using a solution of borohydride-treated mestranol at a concentration of 38·6 μg/ml, equal extinction points at 273 mμ and 288 mμ were chosen; at these wavelengths the absorption was 0·76 (fraction F) of the absorption at the peak of 278 mμ. Substitution of these data in the above equations and solving for x and y is shown as follows:

$R = 0{\cdot}180 \qquad Q = 0{\cdot}237 \qquad a = 5\ m\mu \qquad b = 10\ m\mu$

$$x = (A - C)\frac{10}{15} \qquad \frac{C - y}{B - (x + y)} = \frac{0{\cdot}180}{0{\cdot}237} = 0{\cdot}76$$

$$x = 0{\cdot}667\,A - 0{\cdot}667\,C \qquad y = 2{\cdot}109\,A - 3{\cdot}164\,B + 2{\cdot}054\,C$$

$$\text{Corrected absorption} = B - (x + y)$$
$$= 4{\cdot}164\,B - 2{\cdot}776\,A - 1{\cdot}387\,C$$

Application of this correction to determine the mestranol content of samples of norethynodrel after reduction with borohydride requires that the background absorption due to reduced norethynodrel should be linear over the wavelength range covered by the correction. Direct confirmation of this linearity was not possible as all samples of norethynodrel examined contained 0·5% or more of mestranol. A sample of norethynodrel was subjected to the acid catalysed rearrangement to norethisterone; this latter compound is less soluble than its precursor in polar solvents and recrystallization gave a product much lower in mestranol content. Examination of its ultraviolet spectrum after borohydride reduction (Fig. 2) showed substantial linearity over the range

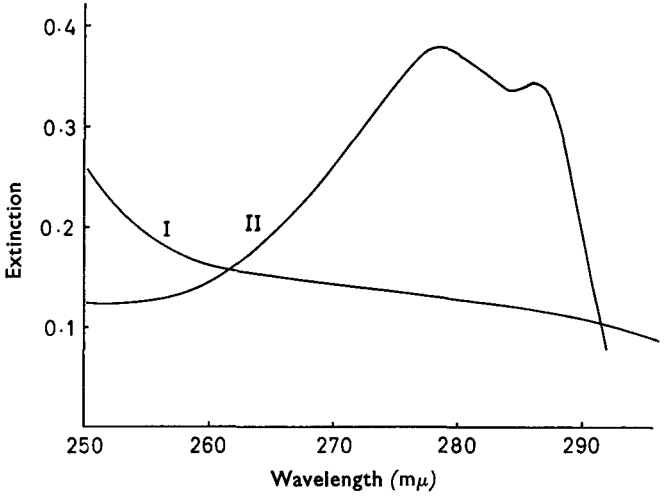

FIG. 2. I. Ultraviolet spectrum of norethisterone after borohydride reduction (137 μg/ml). II. Tablets of mestranol and norethynodrel after borohydride reduction (42 μg mestranol/ml).

260 to 300 mμ. It is believed that the borohydride reduction product of norethynodrel is identical to that of norethisterone.

GENERAL METHOD

On the basis of the foregoing experimental work the following procedure was established for the determination of norethynodrel and mestranol in tablets, alone and in combination.

Norethynodrel. Transfer a weight of powdered tablets equivalent to 10 mg of norethynodrel to a 100 ml volumetric flask and dilute with

methanol (100 ml). Stir for 15 min with a magnetic stirrer, filter and transfer 5 ml of filtrate to a 100 ml volumetric flask, add N hydrochloric acid (5 ml) and heat in a boiling water-bath for 5 min. Cool, dilute to 100 ml with methanol and determine the extinction in a 1 cm cell at the maximum at about 241 mμ against a similarly treated blank.

For calculation use an E (1%, 1 cm) of 560.

Mestranol. Reagents: Potassium borohydride, reagent grade. Methanol reagent, dissolve potassium borohydride (1·5 g) in methanol A.R. (1 litre), add N sodium hydroxide (8 ml), reflux for 4 hr and distil.

Transfer a weight of powdered tablets equivalent to 1·5 mg of mestranol to a 50 ml volumetric flask and add methanol reagent (35 ml). Shake for 30 min, add 0·1 N sodium hydroxide (4 ml) containing potassium borohydride (200 mg) and stand overnight. Add 0·1 N hydrochloric acid (4 ml), shake gently to eliminate dissolved gases and dilute to 50 ml with methanol reagent. Determine the extinction in a 1 cm cell at 273, 278 and 288 mμ, against a similarly treated blank.

Corrected extinction = 4·164 extinction at 278 mμ—2·776 extinction at 273 mμ—1·387 extinction at 288 mμ. For the calculation use an E (1%, 1 cm) of 70.

Results and discussion

Table 1 summarizes results obtained using the method described on tablets in which the excipients and lubricants were lactose, starch, alginic

TABLE 1. RESULTS WITH TABLETS IN WHICH THE EXCIPIENTS AND LUBRICANTS WERE LACTOSE, STARCH, ALGINIC ACID, MAGNESIUM STEARATE AND STEARIC ACID

	Norethynodrel content mg tablet		Mestranol content mg tablet	
Sample tablets	Nominal	Found	Nominal	Found
1	0	—	0·1	0·099
2	0	—	0·1	0·108
3	0	—	0·1	0·103
4	3·50	3·54	0·14	0·12
5	5·00	5·08	0·075	0·073
6	5·00	5·08	0·075	0·076
7	5·00	5·00	0·075	0·079

acid, magnesium stearate and stearic acid. No interference was experienced from these vehicles. The relatively low E (1%, 1 cm) of 70 for mestranol at the wavelength of determination requires the extraction of 1 to 2 g of tablets giving a high level of any methanol soluble substances present in the formulation. The borohydride reduction and subsequent correction procedure are likely to contribute towards the elimination of interference from tablet excipients but it is possible that alternative extraction procedures may be required for other formulations.

References

Chissell, J. F. (1964). *J. Pharm. Pharmac.*, **16**, 490–492.
Legrand, M., Delaroff, V. & Smolik, R. (1958). *Ibid.*, **10**, 683–686.
Morton, R. A. & Stubbs, A. L. (1946). *Analyst*, **71**, 348–356.
Schulz, E. P. (1965). *J. pharm. Sci.*, **54**, 144–147.