Determination of 17α-Ethynylestradiol-3-methyl Ether in Tablets by a Colorimetric Assay

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A colorimetric method has been developed for assaying 17α-ethynylestradiol-3-methyl ether in tablets. The method is based on the formation of a colored complex with phenol–sulfuric acid reagent. This complex exhibits an absorption maximum at 550 mμ, obeys Beer's law, and is stable for a reasonable length of time. Optimum conditions for the color formation in the presence of a nonphenolic steroid have been determined and are reported herein.

ESTROGENS in combination with progestational agents have come into prominence as a contraceptive composition in the last decade. The most frequently used estrogen in these combinations is 17α-ethynylestradiol-3-methyl ether (mestranol). A rapid and sensitive colorimetric determination of mestranol in a tablet combination with norethindrone[1] stored under a variety of conditions is the subject of this paper.

Steroidal estrogens have been assayed by a number of methods. Of these ultraviolet spectrophotometric (1, 2) and spectrofluorometric (3–5) analyses have been preferred by a number of authors. The lack of sensitivity of the ultraviolet method of assay on aged tablets was shown by this laboratory (2).

Recently ion-exchange (6) and paper chromatographic methods (7) have also appeared in the literature. These methods are sensitive but they lack the specificity required in the presence of other steroids for a rapid analytical method. Specificity can be imparted to these methods through prior isolation of the estrogens by other techniques. However, because of the number of manipulations required, varied results may be obtained. Schulz (8) has discussed the difficulties of assaying mestranol by a paper chromatographic method.

Gas-liquid chromatography has also been considered to be a desirable technique (2, 8–10). It offers quantitation with concurrent fractionation. The GLC process developed for assaying mestranol by this laboratory (2), although efficient, was relatively slow for routine assay purposes.

A colorimetric technique was resorted to because of its rapidity and reproducibility. Estrogens have been determined by colorimetric methods using 2,6-dibromoquinone chlorimide (11) and various diazotized amines (12–18) as the color-producing reagents. The official U.S.P. procedure for the determination of estrone, estradiol, and ethynyl estradiol, is based on the Kober reaction.

In this investigation the color development of mestranol was carried out with a phenol–sulfuric acid reagent. The color formation was stable over a reasonable period of time and no interference by nonphenolic steroids was observed. As a result of these studies with extraction procedures and different commercial phenols, a rapid, accurate, and sensitive colorimetric procedure for mestranol in estrogen–progestin tablets was developed. A comparison of the results from the ultraviolet, gas-liquid chromatographic, and colorimetric methods is also presented.

EXPERIMENTAL

A Beckman model DU spectrophotometer was used to determine the absorbance values and a Cary model 11 recording spectrophotometer was used to obtain the spectrum in Fig. 1.

Reagents and Solutions—Freshly distilled phenol, methylene chloride, sulfuric acid (1:1), and methylcyclohexane (spectroquality) are used.

Phenol–Sulfuric Acid Solution—Add 40.0 Gm. of phenol to a 400-ml. beaker containing 100 ml. of 1:1 sulfuric acid solution. Bring the mixture to a boil with continuous stirring and until homogeneous. Store in a volumetric flask and in a dark place.

Mestranol Standard Solution—Weigh accurately 10.0 mg. of mestranol into a 100-ml. volumetric flask and dilute to mark with methylene chloride.

Procedure—Place a number of tablets equivalent to about 400 mcg. of mestranol in a 29.5 × 80-mm. vial. To this add 4.0 ml. of distilled water and shake until the tablets disintegrate. Add 4.0 ml. of methylcyclohexane and shake for 30–60 min. Centrifuge for approximately 2 min. and transfer 1.0 ml. of clear methylcyclohexane solution to a 21 × 50-mm. vial. Also transfer 1.0 ml. of the standard mestranol solution into another 21 × 50-mm. vial. Evaporate the solvent in each vial over a steam bath and under a stream of nitrogen. To each vial add 5.0 ml. of phenol–sulfuric acid solution. To a third 21 × 50-mm. vial add 5.0 ml. of phenol–sulfuric acid solution to serve as a reference. Shake the three vials thoroughly and place them in a 55° water bath for 5 min. Allow the solutions to come to room temperature in a dark place (about 15 min.) and determine the absorbance of the standard and the sample at 550 mμ, using the contents of the third vial as the reference solution.

DISCUSSION

The absorbance spectrum of mestranol resulting from the reaction with phenol–sulfuric acid is shown in Fig. 1. It exhibits broad absorption maxima at 470 mμ and 550 mμ. The stability of the color as measured at 550 mμ depended upon the phenol used. For example, a reagent made with May and Baker phenol gave a stable color over a period of 6 hr., whereas other reagents made from different phenols

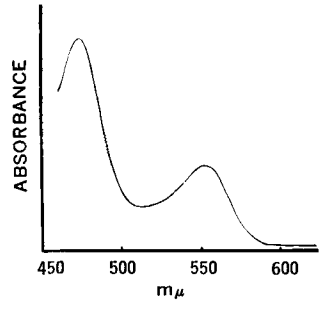

Fig. 1—Visible absorption spectrum of mestranol treated with phenol–sulfuric acid reagent.

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[1] This combination is marketed as Ortho-Novum by Ortho Pharmaceutical Corp., Raritan, N. J.

TABLE I—MESTRANOL ASSAY ON ESTROGEN–PROGESTIN TABLETS

Lot	Storage Conditions	Original Granulation U.V. Assay, mcg./Tab.	U.V. Assay After Storage, mcg./Tab.[a]	Colorimetric Assay, mcg./Tab.	GLC Assay[a]
A	6 wk. 100/80	50.7	39.1	50.3	51.4
B	3 mo. 100/80	51.3	42.8	50.1	47.2
C	6 mo. 37°	80.0	71.7	77.8	84.3
D	7.5 mo. R.T.	49.9	43.6	48.8	51.6
E	12 mo. R.T.	56.0	51.2	58.7	55.1
F	13 mo. R.T.	51.5	45.6	50.6	52.2
G	18 mo. R.T.	57.6	48.9	56.5	56.0

[a] Data obtained from *Reference 2*.

did not give so stable a color. However, in all instances the color intensity remained constant for a sufficient length of time (20–30 min.) to allow recording of the spectrophotometric readings.

The relationship between absorbance at 550 mμ and the quantity of mestranol was found to obey Beer's law. The standard deviation for accuracy and precision was found to be ± 1.6% and ± 1.52%, respectively.

The specificity of the method for mestranol in the presence of norethindrone and other tablet excipients is demonstrated by the results shown in Table I. The excipients used in the formulation of the tablets were lactose, cornstarch, magnesium stearate, and polyvinylpyrrolidine. The data in Table I include several samples of formulation stored under various conditions of temperature and humidity. A previous study (2) indicated that mestranol did not undergo any decomposition under these conditions. Hence, the selectivity and sensitivity of this colorimetric method appeared adequate for performing routine studies.

Table I compares the data obtained by the ultraviolet and gas chromatographic methods (2) with the present colorimetric results. It is evident that the color assay compares favorably with the gas chromatographic assay and is superior to the ultraviolet method. The mechanism by which phenol–sulfuric acid reacts with mestranol and other estrogens to give colored products is under investigation.

SUMMARY

A quantitative colorimetric procedure using phenol–sulfuric acid has been developed for the analysis of mestranol in estrogen–progestin tablets. The accuracy and precision of this method are ±1.6% and ±1.52%, respectively. The nonphenolic steroid and tablet excipients do not interfere in the determination.

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Communications

Identity of Columbianadin and Zosimin

Sir:

Recently, Nikonov and Baranauskaite (1, 2) reported the isolation of a purportedly new coumarin, zosimin, from the roots of *Zosimia absinthifolia* (Vent) Link. This compound analyzed for the formula $C_{19}H_{20}O_5$ and, on alkaline saponification, yielded *cis*-1,2-dimethylacrylic acid (*i.e.*, tiglic acid) and a hydroxylactone, zosimol (m.p. 156–158°), with the formula $C_{14}H_{14}O_4 \cdot {}^1/_2CH_3OH$. It was recognized that zosimin was isomeric with columbianadin (3, 4), but the apparent divergence in optical activities between the two coumarins and the corresponding hydroxylactones led the authors to the conclusion that they were different and that zosimin was, in fact, a new coumarin. Their decision apparently was confirmed by the fact that zosimin yielded tiglic acid on alkaline saponification. The identity of the hydroxylactone was ascertained by Perel'son *et al.* (5) by both spectral and chemical studies, thus eliminating the several other possible structural candidates [*e.g.*, marmesin (6), lomatin (7), 3′-hydroxy-3′,4′-dihydroxanthyletin (8), *etc.*]. On this basis it was concluded originally that zosimin