Gas Chromatographic Determination of Anagestone Acetate in Fertility Control Tablets

By ARVIN P. SHROFF and R. E. HUETTEMANN

A gas-liquid chromatographic technique has been developed for the determination of anagestone acetate in tablets. A 3 percent DEGS column gave good resolution between the peaks of anagestone acetate and internal standard. The accuracy and the precision of the method are 1.97 and 0.052, respectively. Anagestone acetate does not undergo any alteration after 18 months of storage.

THE INCREASING awareness of the population "explosion" has stimulated the development of a number of oral contraceptive agents. Estrogen-progestin combinations are widely used for this purpose. Of the seven U. S. companies which offer eleven different dosage forms, the choice of estrogen is between either 17-α-ethynylestradiol (EE) or 17-α-ethynylestradiol 3-methylether (MEE). The progestational agents, however, vary in their chemical structure but they fall into two main classes. The members of one class are characterized by the absence of the C-19 methyl group and are designated as "19-nor" compounds. The other group is characterized by modification of the basic progesterone structure with different types of substitutions to form the so-called "substituted" progestins.

Estrogens in the presence of some of these progestins have been assayed by colorimetric (1, 2), ultraviolet (3), spectrofluorometric (4), and gas-liquid chromatographic (3, 5, 6) methods.

Similarly, the progestational agents of the "19-nor" and "substituted" progestin classes have been quantitated by various analytical techniques. For example, Bastow quantitated 17-α-hydroxy-19-nor-17-pregn-5(10)-en-20-yn-3-one (norethynodrel) with an ultraviolet spectrophotometric technique (7) and France and co-workers used GLC for 6-α-methyl-6-dehydro-17α-acetoxyprogesterone (6).

In the present investigation we describe the gas-liquid chromatographic determination of a new "substituted" progestin 3-desoxy-17 α-acetoxy-6-α-methylpregn-4-en-20-one(I) (anagestone acetate), in the presence of MEE (II). The determination was carried out on a 3% DEGS coated column using MEE as an internal standard. The steroids do not undergo any decomposition on the stainless steel column when proper conditions are maintained and MEE can be separated without prior acylation or trimethylsilyl etherification.

EXPERIMENTAL

Apparatus and Operational Parameters—An F&M model 1609 instrument equipped with a hydrogen flame-ionization detector and modified injection port was employed. The modified injection port was made by F&M Scientific to eliminate the potential for inlet sample splitting. The recorder was a Minneapolis Honeywell "Electronik" with a 1-mv. full-scale deflection and was operated at a chart speed of 15 in./hr. A coiled stainless steel column 39.4 in. long and 1/4 in. i.d. packed with 3% DEGS on Gas Chrom Z (100–120 mesh) was used. The column packing was prepared by the evaporation technique. The packed column was conditioned at 215° for 24 hr. with nitrogen flowing at 63 ml./min. All samples were injected by means of a 10-μl. Hamilton syringe. The operational parameters were: column temperature 215°; inlet-port temperature 235°, and detector temperature 250°. Nitrogen was used as a carrier gas flowing at 65 ml./min. Oxygen and hydrogen were maintained at about 400 ml./min. and 45 ml./min., respectively.

Standard MEE Solution—Weigh accurately 300 mg. of reference standard MEE (E 1%, 1 cm. 60–65) into a 100-ml. volumetric flask and dilute to mark with methylene chloride.

Standard Anagestone Acetate Solution—Place exactly 100 mg. of anagestone acetate into a 100-ml. volumetric flask and dilute to mark with methylene chloride.

Preparation of a Typical Standard Curve—Pipet into three different vials 1.0 ml., 2.0 ml., and 3.0 ml., of the standard anagestone acetate solution and evaporate to dryness on a steam bath and under a stream of nitrogen. Dissolve each residue with 1.0 ml. of standard MEE solution (internal standard) and inject approximately 2 μl. into the gas chromatograph. Adjust the attenuator to 320 and elute for 20 min. The first major peak is attributed to anagestone acetate and the second major peak to MEE. The areas are calculated, after drawing a tangent to both minima of each peak, by multiplying the height of the peak by the width at one-half the peak height. A plot of the ratios of areas under anagestone acetate and MEE *versus* the weight of anagestone acetate is obtained and a best straight line drawn. All the points on the curve are obtained in triplicate.

Analysis of Tablets—Two tablets (2 mg. anagestone acetate and 80 mcg. MEE per tablet) equivalent to 4.0 mg. of anagestone acetate are placed in a 21 × 50-mm. vial and treated with 2.0 ml. of distilled water and 2.0 ml. of cyclohexane. The vial is clamped to a Burrell wrist-action type shaker and allowed to shake vigorously for 0.5 hr., then centrifuged for 1 min. and 1.0 ml. of the upper phase is pipeted into another 21 × 50-mm. vial. This solution is evaporated on a steam bath and under a stream of nitrogen. The residue thus obtained is redissolved in 1.0 ml. of standard MEE solution (internal standard) and a 2-μl. injection made into the gas chromatograph. The attenuation and calculations are similar to "preparation of typical standard curve." The amount of anagestone acetate is obtained from the standard curve.

DISCUSSION AND RESULTS

Theoretically each combination tablet contains 2 mg. of anagestone acetate and 80 mcg. of MEE along with other excipients of the tablet. The anagestone acetate present is 25 times in excess of the MEE.

The efficiency of using MEE as an internal stan-

Received November 17, 1967, from the Analytical Research Group, Division of Organic Chemistry, Ortho Pharmaceutical Corporation, Raritan, NJ 08869
Accepted for publication January 8, 1968.

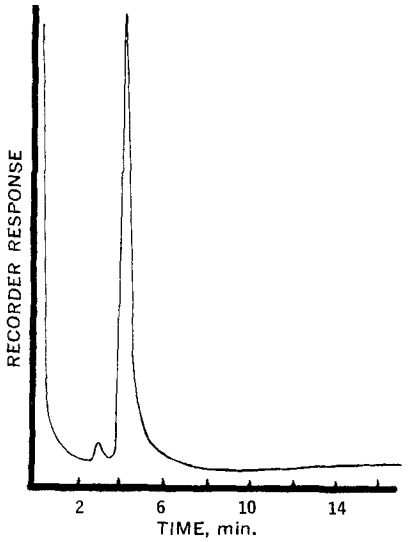


Fig. 1—Gas chromatogram of an extract from combination tablet showing dihydro derivative and anagestone acetate.

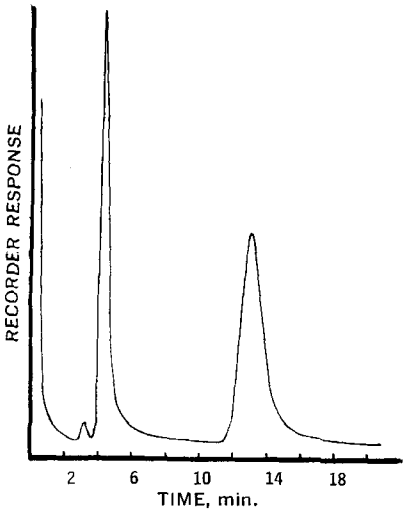
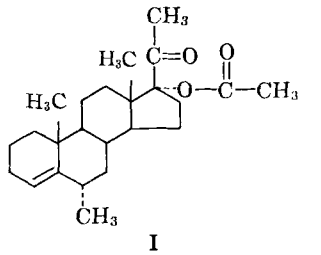


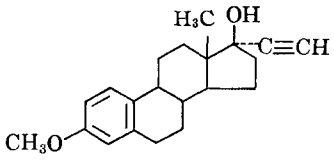
Fig. 2—A typical gas chromatogram of a tablet extract to which internal standard (MEE) has been added.

dard was checked by extracting two tablets with water–cyclohexane and following the procedure outlined in the experimental section but omitting the internal standard. A typical gas chromatogram of an extract from combination tablets is shown in Fig. 1. The small peak preceding the major anagestone acetate peak is attributed to a dihydro derivative of anagestone acetate (a synthetic by-product). The peak response for MEE (see Fig. 2 for retention time) was negligible, if any, with the attenuation used. The gas chromatogram of material extracted from tablets to which the internal standard (MEE) was added is shown in Fig. 2. The internal standard does not interfere with detection of anagestone acetate and the resolution between the peaks is good.

The MEE showed no decomposition to estrone methyl ether on a freshly prepared column. However, if the column packing was not prepared properly, then after a few days and, at times, after conditioning the column decomposition became apparent. The peak for estrone methyl ether appeared between anagestone acetate and MEE (Fig. 3) and it was found that this decomposition did not vary with time. Thus, by preparing a fresh calibration curve the column can be used effectively. Routinely we have found the method to be independent of the volume of the injected sample and independent of small variations in column temperature and gas flow.



I
Anagestone Acetate



II
MEE

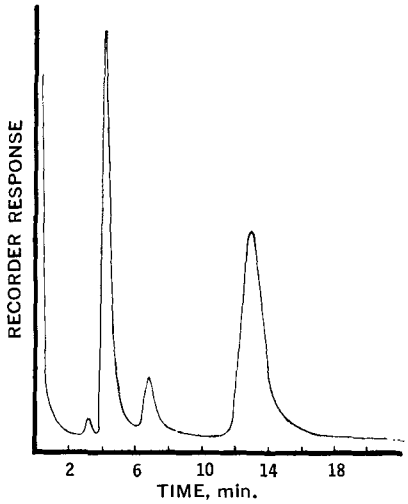


Fig. 3—Gas chromatogram showing the decomposition of MEE to estrone methylether.

The ratio of peak areas obtained for different concentrations of anagestone acetate in the standard solutions is given in Table I. A plot of these data

TABLE I—CONCENTRATION *Versus* RATIO OF PEAK AREAS

No.	Anagestone Acetate	Ratio of Peak Areas[a]
1	1.0	0.282
2	2.0	0.590
3	3.0	0.879

[a] Average of three determinations.

TABLE II—ACCURACY DATA FOR THE GLC METHOD

No.	Mg. Added	Mg. Found[a]	% Recovery ($\bar{x}$)[b]
1	2.0	1.95	97.5
2	2.0	1.96	98.0
3	2.0	2.02	101.0
4	2.0	2.04	102.0
5	2.0	2.01	100.5

[a] $\sigma = \pm 1.97\%$. [b] $\bar{x} = 99.8$.

TABLE III—REPRODUCIBILITY DATA FOR THE ASSAY METHOD ON FRESH TABLETS[a]

No.	Mg. Found (x)	$(\bar{x} - x)^2$
1	2.04	0.0009
2	1.96	0.0025
3	1.96	0.0025
4	2.08	0.0049
5	2.01	0.0000

$\sigma = \pm 0.052$

[a] Each tablet contains theoretical amount of 2 mg. of anagestone acetate.

is a straight line (standard curve). The accuracy of the GLC method was obtained from the data in Table II. The known and found values along with the sigma value (1.97) show that the analysis can be performed satisfactorily. The precision ($\sigma = 0.052$) studies were carried out on fresh tablets and indicated (Table III) the method was sensitive and can be adopted for routine analysis.

Several batches of tablets stored at various temperature stations for different lengths of time were assayed. The results, recorded in Table IV, demonstrate that over a period of 18 months, anagestone acetate does not undergo any alteration.

TABLE IV—ASSAY DATA ON COMBINATION TABLETS[a] FOR ANAGESTONE ACETATE

No.	Storage Conditions[b]	Mg. Anagestone Acetate per Tablet	% Recovery
1	Fresh	1.96	98.0
2	RT, 3 weeks	2.03	101.5
3	RT, 1 month	1.98	99.0
4	RT, 2 months	2.05	102.5
5	RT, 15 months	2.00	100.0
6	RT, 16 months	1.97	98.5
7	47°, 15 months	2.02	101.0
8	37°, 18 months	2.01	100.5
9	50°, 3 months	2.04	102.0

[a] Each tablet contains theoretically 2.0 mg. of anagestone acetate. [b] RT refers to room temperature.

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Keyphrases

17-α-ethynylestradiol 3-methylether—anagestone acetate tablets
Anagestone acetate—analysis
GLC—analysis

The Analgesic, Hypothermic, and Depressant Activities of Some *N*-Substituted α-5,9-Dimethyl-6,7-benzomorphans

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A series of *N*-substituted α-5,9-dimethyl-6,7-benzomorphans bearing *N*-2-hydroxyalkyl and *N*-2-bromoalkyl substituents have been synthesized and their analgesic, hypothermic, and depressant activities determined. The analgesic activities were not outstanding but the *N*-2-bromoethyl- and the *N*-2-bromopropyl derivatives produced prolonged hypothermia and depression. Possible mechanisms of these prolonged actions are discussed.

It is generally assumed that narcotic analgesics produce their pharmacological effects by interaction at a specific analgesic receptor (or receptors). Extensive work by Beckett and co-workers (1, 2) has resulted in the publication of a tentative description of the analgesic receptor. This description has recently been critically discussed by Portoghese (3) who points out that the analgesic receptor may be more flexible in its binding capability than Beckett originally assumed. Nevertheless, both Beckett and Portoghese assume that one of the principal binding sites at the analgesic receptor is an anionic grouping which is capable of binding, *via* an ion–ion interaction, a protonated amino group of the analgesic molecule.

An anionic binding site (a carboxylate or phosphate anion) is also assumed to be an important fea-

Received February 9, 1967, from the Department of Biochemical Pharmacology, School of Pharmacy, and The Theoretical Biology Center, State University of New York at Buffalo, Buffalo, NY 14214

Accepted for publication January 24, 1968.

This work was supported by grants from the United States Health Foundation of Western New York (G-65-UB-3) and the U. S. Public Health Service (GM11603) with supplemental assistance from NASA (grant No. NGR-33-015-016).