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Note

High-performance liquid chromatographic analysis of the by-products of the synthesis of ethynylestradiol, mestranol, 17α-hydroxyprogesterone caproate and 17α-hydroxy-6-dehydroprogesterone acetate*

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Purity control of drugs is of great importance because the presence of by-products may induce undesirable and sometimes serious complications[1–3]. Usually the acceptable level of impurities in drugs varies from 0.5 to 1.5%. For the detection of minor components and their correct determination it is necessary to have them available in the pure state.

They can be obtained either by synthesis or by isolation from mother liquors during hormone purification. For the isolation, identification and accumulation of minor components from mother liquors we have used different variants of liquid chromatography. The preliminary determination of the components was carried out by thin-layer chromatography (TLC) and the final determination by liquid chromatography.

Preparative high-performance liquid chromatography (HPLC) represents a fast and simple method for the separation and isolation of impurities. Approximately 20 min are required for a 5–10-g sample of a mother liquor, but as the concentration of minor compounds in the mother liquor of drugs is less than 1%, their accumulation requires repetition of the process. After confirmation of the structures of all isolated minor components by physico-chemical methods (mainly by mass, NMR, IR and UV spectrometry), the determination of impurities in the drugs was carried out.

EXPERIMENTAL

As standards of estrogen drugs we used samples specified as "chemical reference substances" in the *Pharmacopoeia of the G.D.R.* (6th edition), the *Compendium*

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Medicamentorum CMEA and the *International Pharmacopoeia* of the World Health Organization (3rd edition), and also the samples from laboratory preparations[4].

A Waters Model Prep 500 liquid chromatograph equipped with a Prep Pak silica column (300 × 57 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.) and a refractometric detector was used. Samples were injected onto the column with a 10-ml syringe. Also, a DuPont Model 830 liquid chromatograph equipped with a Model 837 spectrophotometer and a column with either Zorbax-Sil, Silasorb 600, LiChrosorb Si 60 (250 × 22.7 mm I.D.), Zorbax-Sil or Zorbax CN (250 × 4.6 mm I.D.) was used. Samples were injected onto the column using a Model 7120 syringe-loading injector (0.5 μl) (Rheodyne, Cotati, CA, U.S.A.) with a sample loop of 20–200 μl. For calculations we used a Model 8830 A integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

All reagents were of analytical-reagent grade and solvents were purified by distillation. Under the above conditions we examined a series of steroid drugs, namely ethynylestradiol, mestranol, 17α-hydroxyprogesterone caproate and 17α-hydroxy-6-dehydroprogesterone acetate (sources of these drugs were given previously[5,6]).

The purification and isolation of the contaminants in the steroid drugs were carried out as follows. First, the content of steroids in a mother liquid or sample was determined by TLC on Silufol UV-254 plates with *n*-hexane–acetone (7:3) as mobile phase[4].

The substance to be investigated was dissolved in the mobile phase used and subjected to preparative or semi-preparative HPLC. From ethynylestradiol (mother liquor, 6.5 g) we isolated seven fractions by preparative HPLC on a Waters Prep 500 chromatograph with ethyl acetate–chloroform (5:95) as mobile phase at a flow-rate of 200 ml/min and IR detection (see Fig. 1): I, 0.10 g; II, 4.11 g; III, 1.23 g; IV, 0.11 g; V, 0.14 g; VI, 0.18 g; VII, 0.36 g.

The semi-preparative HPLC separation of these fractions in different solvents, independent of the polarity, we isolated ten compounds, which physico-chemical analysis showed to have a steroidal structure. Other samples were examined using this procedure[5,6].

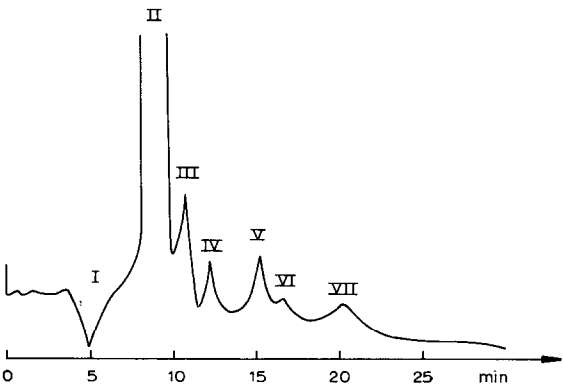

Fig. 1. Preparative separation of a mother liquor sample of ethynylestradiol on a Waters Prep 500 chromatograph with a Prep Pak silica column (300 × 57 mm I.D.) with ethyl acetate–chloroform (5:95) at a flow-rate of 200 ml min and IR detection.

TABLE I

IMPURITIES ISOLATED FROM MOTHER LIQUOR OF ETHYNYLESTRADIOL

No.	*Compound*	*R_F in n-hexane–acetone (7:3)*	*Mol. weight*	*UV maximum (nm)*	*Approximate concentration (%)*
1	17α-Isobutylestradiol	0.12	328	279	0.67
2	Steroid of unidentified structure, $C_{24}H_{34}O_4$	0.18	386	280	0.70
3	Ethynylestradiol 17-methyl ether	0.20	310	264	2.00
4	16-Methoxyethynylestradiol	0.23	310	260	2.00
5	Estradiol	0.29	272	280	0.80
6	Steroid of unidentified structure, $C_{21}H_{26}O_2$	0.30	310	266	0.10
7	Estrone	0.34	270	280	1.00
8	Estradiol 3-methyl ether	0.39	286	278	0.80
9	Mestranol	0.41	310	280	0.57
10	Estrone 3-methyl ether	0.53	284	278	1.00

RESULTS AND DISCUSSION

We found ten minor compounds (with steroidal structures) in ethynylestradiol (Table I). The presence of the same minor components in all the samples of drugs analysed (commercial samples from different firms) showed that they had been prepared in a similar manner.

Compounds 2 and 6 were analysed by mass and NMR spectrometry and were proved to have a steroidal skeleton. In order to verify the hypothesis of a steroidal structure, quantitative reactions of steroid estrogen were conducted in the usual way.

Typical chromatograms are shown in Figs. 2–4.

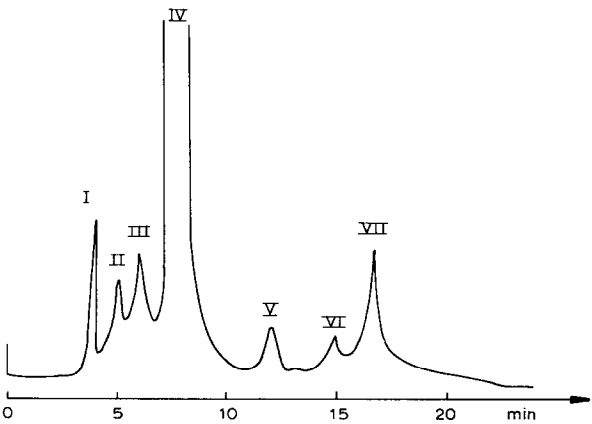

Fig. 2. Semi-preparative separation of the third fraction (see Fig. 1) on a DuPont 830 chromatograph with a LiChrosorb Si 60 column (250 × 22.7 mm I.D.) with ethyl acetate–*n*-hexane (5:95) at a flow-rate of 13 ml min and UV detection at 254 nm.

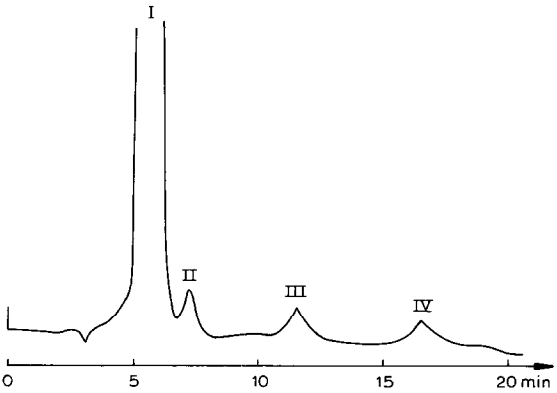

Fig. 3. HPLC trace of 17α-hydroxyprogesterone capronate obtained on a DuPont 830 chromatograph with a Silasorb 600 (250 × 4.6 mm I.D.) with ethyl acetate–*n*-hexane (20:80) at a flow-rate of 1.2 ml min and UV detection at 254 nm.

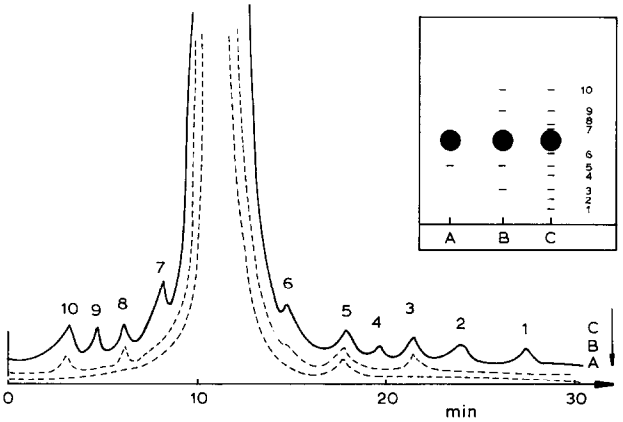

Fig. 4. Detection of impurities in different samples of ethynylestradiol (using markers isolated from the mother liquor product of ethynylestradiol) by means of TLC and HPLC. (A) Farmacon product; (B) Akrichin product; (C) mother liquor product. 1–10 = Compounds identified (see Table I).

CONCLUSIONS

It has been shown that ethynylestradiol (and also mestranol) contain more than ten, 17α-hydroxyprogesterone acetate more the six and progesterone and 17α-hydroxyprogesterone caproate three minor steroidal compounds[5,6].

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