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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF STEROIDAL DRUGS

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SUMMARY

The high-performance liquid chromatographic (HPLC) analysis of progesterone, oxyprogesterone capronate and dehydroacetoxyprogesterone has been carried out successfully using so-called "test impurities". The quantitative and qualitative analysis of related compounds was studied by normal-phase HPLC with a UV detector (254 nm) using ethyl acetate–chloroform (98.5:1.5) and ethyl acetate–hexane (20:80) as solvent systems. Preparative separations of impurities by HPLC were investigated, subsequent structural identification being achieved by mass, UV and IR spectrometry. The method may be applied to the control of the quantitative purity of steroids in drugs and complex mixtures.

INTRODUCTION

Steroids are widely used in medicine and the control of the purity of steroidal drugs is a difficult problem. There are many publications on the resolution and separation of steroids by high-performance liquid chromatography (HPLC), but there is little information on the separation of related compounds and impurities with similar structures and chromatographic behaviour.

In this work we determined the structures and concentrations of related steroids in samples of progesterone, oxyprogesterone capronate and dehydroacetoxyprogesterone. As standards we used samples specified as "chemical reference substances" in the Pharmacopoeia of the G.D.R. (6th Edition), the Compendium Medicamentorum CMEA and the International Pharmacopoeia of the World Health Organization (3rd edition).

Thin-layer chromatography (TLC) is widely used in pharmacopoeial methods for semi-quantitative assays, assuming the same UV sensitivity for both both the main compounds and the impurities; otherwise this may lead to erroneous conclusions about the composition of the sample. We have devised a scheme involving preliminary evaluation of the quality of the samples by TLC, then preparative HPLC for the collection and analysis of the impurities, followed by structural analysis by UV, IR and mass spectrometry. Quantitative compositions of accompanying compounds in the steroid samples were established.

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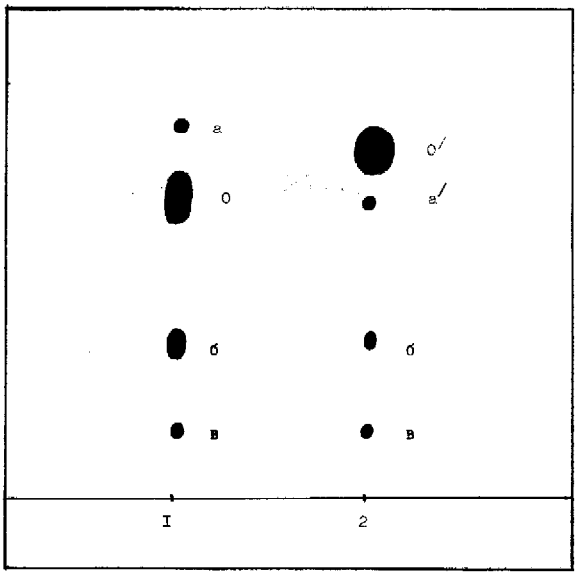


Fig. 1. TLC of progesterone and oxyprogesterone capronate: O = progesterone; O′ = oxyprogesterone capronate; a = product of condensation of progesterone with cyclohexanone; a′ = progesterone; σ = 6β-oxyprogesterone; B = 7α-oxyprogesterone. Silufol UV-254 plate; mobile phase, 30% ethylacetate in chloroform; UV detection at 254 nm.

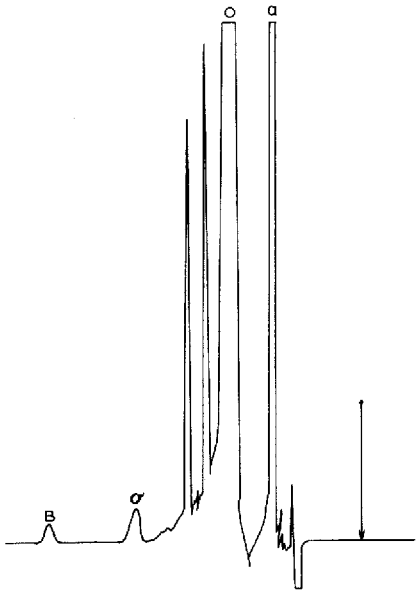
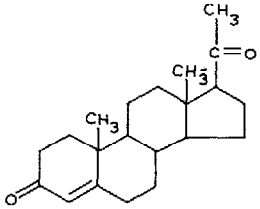
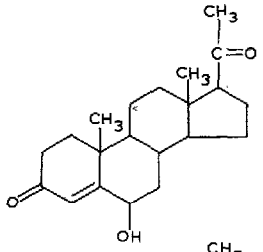
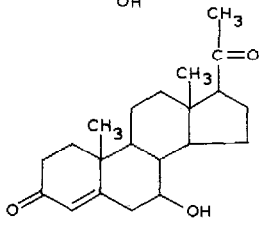
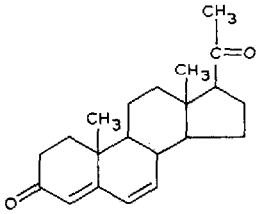
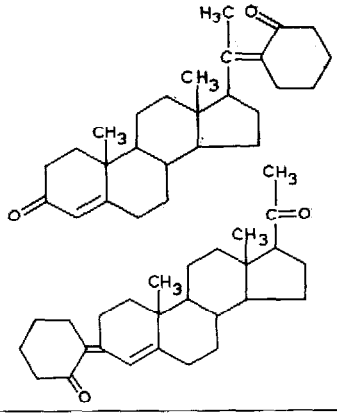


Fig. 2. HPLC of progesterone. Silasorb 600 column; mobile phase, 20% ethylacetate in hexane; flow-rate, 1.2 ml/min; UV detection at 254 nm. Symbols as in Fig. 1.

TABLE I

MAIN CHARACTERISTIC IONS IN THE MASS SPECTRUM OF PROGESTERONE AND RELATED COMPOUNDS

Compound	*Structural formula*	*m/z of characteristic ions*
Progesterone		M^+ 314, M − CH_3^+ 299, M − CO^+ 286, M − CH_3CO^+ 271, M − $CH_2{=}O^+$ 272, and also ions with *m/z* 93, 117, 143, 171, 157, 221, 254, 229
6β-Oxyprogesterone		M^+ 330, M − CH_3^+ 315, M − CO^+ 302, M − CH_3CO^+ 287, M − CH_2CO^+ 286, M − $C_4H_9CO^+$ 245, and also ions with *m/z* 134, 160, 196, 170, 237
7α-Oxyprogesterone		M^+ 330, M − CH_3^+ 315, M − CO^+ 302, M − CH_3CO^+ 287, M − $CH_2{=}CO^+$ 286, M − $C_4H_9CO^+$ 254, and also ions with *m/z* 237, 117, 213, 160, 170
Dehydroprogesterone		M^+ 312, M − CH_3^+ 297, M − CO^+ 284, M − CH_3CO^+ 269, M − $CH_2{=}CO^+$ 270, M − $C_4H_9CO^+$ 227, and also ions with *m/z* 219, 141, 171, 132, 180
Products of condensatione of progesterone with cyclohexanone		M^+ 394, M − CH_3^+ 379, M − CO^+ 366, M − CH_3CO^+ 351, M − $C_4H_9CO^+$ 309, and also ions with *m/z* 173, 197, 223, 221, (substitution in ring) M^+ 394, M − CH_3^+ 366, M − CO^+ 379, M − CH_2CO^+ 352, and also ions with *m/z* 348, 117, 143, 301, 277, 251

(*Continued on p. 140*)

TABLE I *(Continued)*

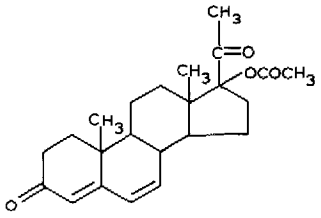
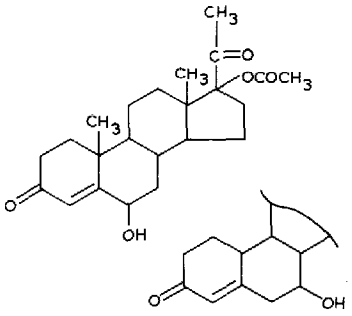
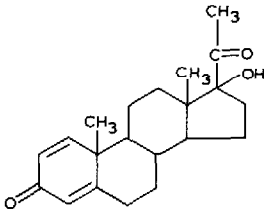
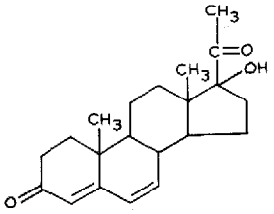
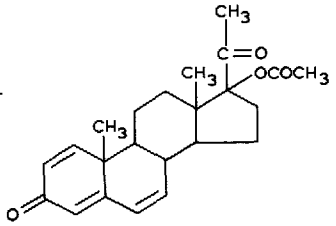
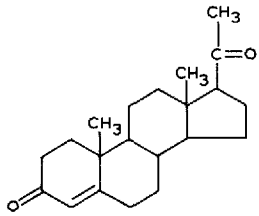
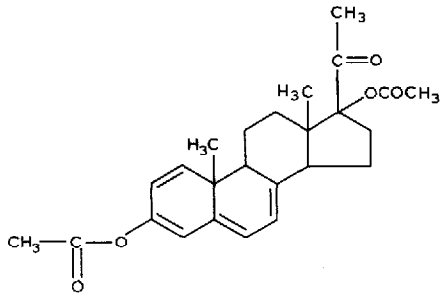
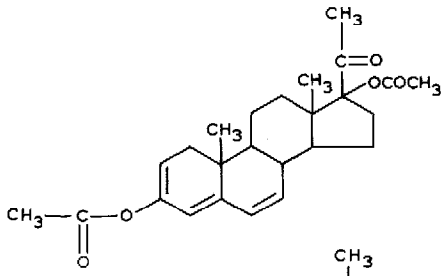
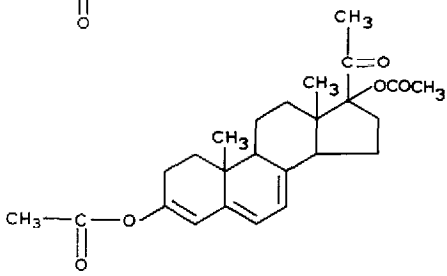
Compound	*Structural formula*	*m/z of characteristic ions*
Dehydroacetoxy-progesterone		M^+ 370, M − CH_3^+ 355, M − CO^+ 342, M − CH_3CO^+ 322, M − $CH_2{=}CO^+$ 328, and also ions with *m/z* 144, 246, 141, 272, 155, 247, 234
6,7(α,β)-Oxy-acetoxyprogesterone		M^+ 388, M − CH_3^+ 373, M − CO^+ 360, M − CH_3CO^+ 345, M − $CH_2{=}CO^+$ 346, and also ions with *m/z* 296, 117, 272, 177, 212, 144, 255, 134, 245
17α-Oxy-$\Delta^{1,4}$-pregnadiene-3,20-dione		M^+ 328, M − CH_3^+ 313, M − CO^+ 300, M − CH_3CO^+ 286, M − $CH_2{=}CO^+$ 285, and also ions with *m/z* 141, 187, 237, 115, 213, 102, 226, 235
17α-Oxy-$\Delta^{4,6}$-pregnadiene-3,20-dione		
17α-$\Delta^{1,4,6}$-Pregnatriene-3,20-dione		M^+ 326, M − CH_3^+ 311, M − CO^+ 298, M − CH_3CO^+ 284, and also ions with *m/z* 144, 182, 235, 156, 170, 139, 187

TABLE I *(Continued)*

Compound	*Structural formula*	*m/z of characteristic ions*
Progesterone		M^+ 314, M − CH_3^+ 299, M − CO^+ 286, M − CH_3CO^+ 271, M − $CH_2{=}CO^+$ 272, M − $C_4H_9CO^+$ 229, and also ions with *m/z* 143, 171, 221, 157, 254, 117, 93
Enol acetates		M^+ 418, M − CH_3^+ 403, M − CO^+ 375, and also ions with *m/z* 144, 274, 156, 262, 180, 238
		M^+ 420, M − CH_3^+ 405, M − CO^+ 392, M − $CH_2{=}CO^+$ 377, and also ions with *m/z* 361, 144, 276, 134, 286, 184, 236
		M^+ 420, M − CH_3^+ 405, M − CH_3CO^+ 377, and also ions with *m/z* 144, 276, 156, 264, 180

EXPERIMENTAL AND RESULTS

TLC was carried out on Silufol UV-254 plates using chloroform–ethyl acetate (7:3) as the mobile phase[5]. Amounts of about 100 μg of samples were applied to the plates with UV detection at 254 nm; spots with R_F values of 0.56, 0.21 and 0.08 were observed. The results are shown in Fig. 1.

It has been shown earlier[2,7,8] that synthons and intermediate compounds were

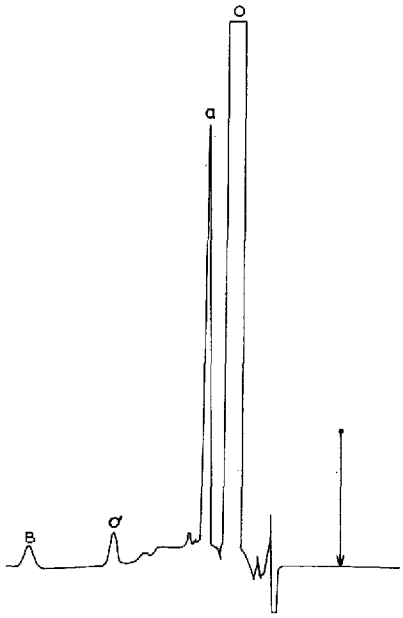


Fig. 3. HPLC of oxyprogesterone capronate. Conditions as in Fig. 1.

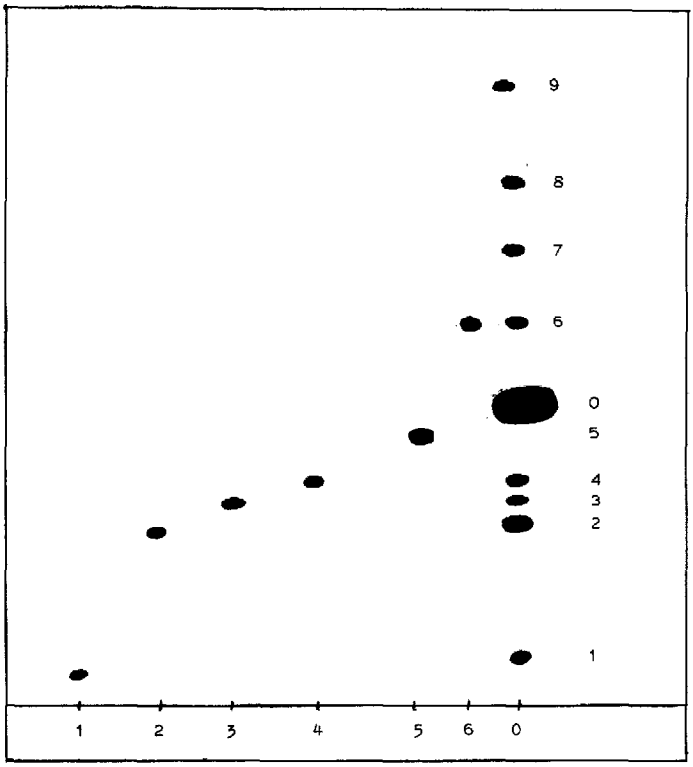


Fig. 4. TLC of dehydroacetoxyprogesterone: 0 = dehydroacetoxyprogesterone; 1 = 6β-oxyacetoxyprogesterone; 2 = 7α-oxyacetoxy progesterone; 3 = 17α-oxy-$\Delta^{1,4}$-pregnadiene-3,20-dione; 4 = 17α-$\Delta^{1,4,6}$-pregnatriene-3,20 dione; 5 = 17α-oxy-$\Delta^{4,6}$-pregnadiene-3,20-dione; 6 = progesterone; 7–9 = enol acetates. Silufol UV-254 plate; mobil phase, 30% ethylacetate in chloroform; UV detection at 254 nm.

absent from final products. This may be a result of the termination processes or the purification of the samples. We therefore subjected the samples to HPLC on a Du-Pont 830 chromatograph with a 250 × 4.6 mm I.D. column of Silasorb 600 (5 μm) and UV detection at 254 nm. The mobile phase consisted of 20–40% of ethyl acetate in hexane at a flow-rate of 1.2 ml/min over 20 min using an HP-3380A integrator. Three impurities and two supplementary compounds were detected after the peak of the main substance (see Fig. 2). For the determination and identification of the structures of the unknown substances, detected by TLC and HPLC, the five fractions prepared by these methods were examined by UV, IR and mass spectrometry[1,4,6]. Table I shows the mass spectral data for progesterone and its impurities. The results suggest that the impurites are 6-dehydroprogesterone and 7α- and 6β-oxyprogesterone. The substance with M^+ 312, IR 1700–1600 cm^{-1} and UV absorption maximum at 280 nm was identified as 6-dehydroprogesterone[3,4,6]. Its structure was confirmed by synthesis. The resolution 6-dehydroprogesterone and progesterone cannot be achieved by TLC using the usual solvent systems. 7α- and 6β-oxyprogesterone were identified by mass spectrometry (M^+ 330), IR (3400 cm^{-1}, characteristic band of the OH group) and UV absorption maximum at 241 nm. TLC showed two less polar zones. The IR and mass spectrometric data indicated mixtures of products of the condensation between progesterone in the 3- and 20-positions and the cyclohexanone which was used at the final stages of the synthesis of the progesterone. These compounds were detected by TLC as being more polar than progesterone, and we have not identified impurities more polar than 6-dehydroprogesterone by HPLC.

Oxyprogesterone capronate and dehydroacetoxyprogesterone were also studied. Progesterone and 7α- and 6β-oxyprogesterone were detected in oxyprogesterone capronate (see Figs. 1 and 3 and Table I). Results of analysis of dehydroacetoxyprogesterone are shown in Figs. 4 and 5 and Table I.

These studies will be extended to the analysis of other steroidal drugs.

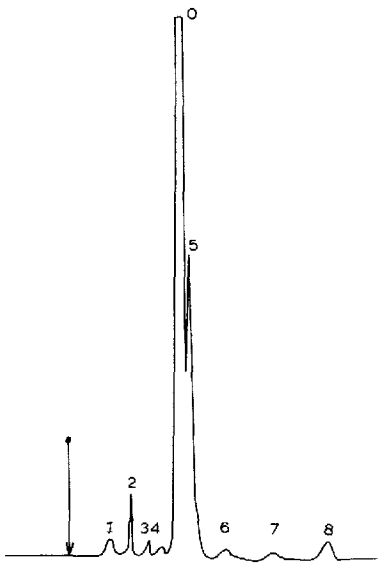


Fig. 5. HPLC of dehydroacetoxyprogesterone. Conditions as in Fig. 4.

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