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Note

Analysis of steroids

XXX*. Simultaneous determination of α- and β-ethynodiol diacetate by high-pressure liquid chromatography

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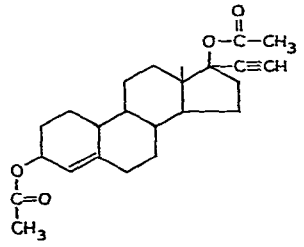
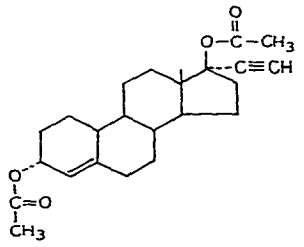
One of the most critical steps in the synthesis of the important gestogenic hormone, ethynodiol diacetate (EDDA) is the reduction of the 3-keto group[1,2]. In addition to the β-epimer (β-EDDA), a small quantity of α-EDDA is also formed, which is therefore the most likely impurity of β-EDDA.



β-EDDA



α-EDDA

Several methods have been described for the determination of EDDA, among them titrimetric[3–6], spectrophotometric[4,5,7], colorimetric[1,8–10], and fluorimetric[1] procedures. However, none of them appears to be suitable for the determination of the ratio of the α- and β-epimers. Gas chromatography does not seem to be suitable for this purpose either, as EDDA decomposes under these conditions[11]. Quite recently, Csizér and Görög[12] described the simultaneous determination of the epimers by spectrophotometric-differential kinetic method, but this method can be used only if the relative quantity of the α-epimer exceeds 15%. The aim of this study was to develop a sensitive high-performance liquid chromatographic method, enabling the determination of as little as 0.5% of α-EDDA.

* Part XXIX: *Zbl. Pharm.*, 116 (1977) 259.

EXPERIMENTAL

A Hewlett-Packard 1010B high-pressure liquid chromatograph was used equipped with a variable-wavelength UV detector and Hewlett-Packard 3380 integrator.

The separation was carried out at ambient temperature, using a 30 cm × 4 mm μBondapak C_{18} (Waters) column and a mixture of methanol and water (4:1) as the eluent at a flow-rate of 1.5 ml/min. The chromatograms were monitored at 210 nm. 25 μl of the methanolic test solution containing 25 μg of EDDA was injected into the chromatograph, using a Valco loop injector.

RESULTS AND DISCUSSION

Both epimers of EDDA have a UV absorption maximum at 204 nm, due to their isolated double bonds. The end absorption occurs at about 235 nm. Setting the wavelength to 204 nm would result in high sensitivity, but a noisy baseline. At 210 nm, which is on the slope of the spectrum, sufficiently sensitive detection and stable baseline could be achieved.

Fig. 1 shows the chromatogram of a test mixture containing equal quantities of the epimers. As can be seen, the retention times are 15.2 min for α-EDDA and 17.2 min for β-EDDA, allowing separate quantitative analysis.

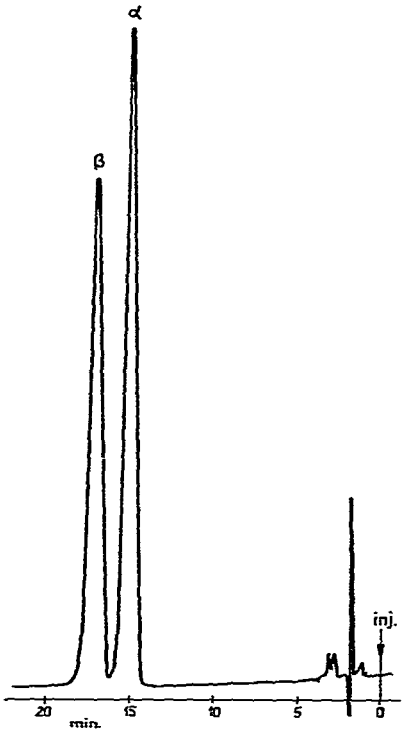


Fig. 1. Chromatogram of a model mixture containing equal quantities of α- and β-EDDA. For the chromatographic conditions see Experimental section.

The results of model experiments are summarized in Table I. As little as 0.5% of α-EDDA can be determined with acceptable precision. The percentage of α-EDDA is calculated using the following equation

$$\% = \frac{I_\alpha}{I_\alpha + 1.09 \cdot I_\beta}$$

where I_α and I_β are the integrator readings for α-EDDA and β-EDDA, respectively, and the constant is due to the slight difference between the absorptivities of the epimers at 210 nm.

TABLE I

MODEL EXPERIMENTS. DETERMINATION OF α-EDDA IN EDDA

Percentage of α-EDDA		*Relative standard deviation (±%)* *
Taken	*Found*	
10.0	10.4	0.75
5.0	5.3	1.7
2.0	2.11	2.6
1.0	0.96	2.5
0.5	0.44	4.3

* Six replicate analyses.

It is worth mentioning that under the described chromatographic conditions some other potential impurities of β-EDDA possess the following retention times: norethisterone, 3.7 min; norethisterone acetate, 5.5 min; ethynodiol, 4.7 min; ethynodiol 3-acetate, 9.1 min; ethynodiol 17-acetate, 6.4 min; 17-ethynyl-3,5-oestradiene-17-ol acetate, 25.8 min.

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