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Anomalous Epimerization of Estriol to 16-Epiestriol

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The epimerization of estriol (1,3,5,(10)-estratrien-3,16α,17β-triol) to 16-epiestriol (1,3,5,(10)-estratrien-3,16β,17β-triol) as a result of undetermined catalysis is described. During the course of estrogen studies in these laboratories, the appearance of abundant quantities of an impurity was noted in what had previously seemed to be essentially homogeneous solutions of estriol. The substance was found to be present in quantities of less than 1% when crystalline estriol first was dissolved in 95% ethanol, but within six months it had increased to form more than 95% of the total steroid content in solutions kept refrigerated. The transformation was more rapid at room temperature. The laboratory conditions during which this epimerization takes place, and the chromatographic and special properties used to establish the identity of 16-epiestriol are presented in this paper.

MATERIALS AND METHODS

Crystalline estriol and 16-epiestriol were obtained from Mann Research Laboratories, New York, New York, and Steraloids, Pawling, New York. These materials were dissolved in redistilled 95% ethanol (U. S. Industrial Chemical U.S.P.) in solutions of concentration of 1 mg/ml and kept in the refrigerator (4–5°C) or at room temperature (23°C) in a room well lit by fluorescent lamps.

Purity was determined by thin-layer chromatography and confirmed by infrared spectra. Reference 16-epiestriol was about 95% pure when received and after purification by thin-layer chromatography it was more than 99% pure.

Chromatography. The steroids were separated by thin-layer chromatography using silica gel H (Merck) 0.25 mm thick on glass plates 20 $\times$ 20 cm in size. Free steroids were separated in 15% ethanol in benzene, and in 50% ethyl acetate in methylene chloride. Estriol and 16-epiestriol separate well in these systems (Table 1). The acetates were

separated in 15% isopropyl ether in benzene and 0.75% ethyl acetate in methylene chloride. The acetates of estriol and 16-epiestriol are well resolved in these systems (Table 1). The spots were identified by spraying the plates with 20% *p*-toluenesulfonic acid (1).

The steroids were also identified by paper chromatography using the methylene chloride/ethylene glycol system (2) and by gas-liquid chromatography (3). For the latter analysis a combination column of 10% QF-1 (fluorosilicone, Dow-Corning) and 5% L-45 (methylsilicone, General Electric) was used under the following conditions: column temperature, 240°; flash temperature, 270°; and detector temperature, 240°C. Inlet pressure of argon carrier gas was 30 psi. A Chromalab model A-110 (Glowall Corp., Willow Grove, Pa.) equipped with a 6 ft × 4 mm glass coil column and a 1 cm radium foil Lovelock detector was used.

Spectral Properties. Colorimetric analysis was performed using a modified Kober-Ittrich procedure (4). The absorption spectra of the steroids in sulfuric acid also were determined. In addition, infrared absorption of crystalline steroids in a KBr pellet were studied. The instrument used for the latter analysis was the Perkin-Elmer model 237B.

Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

RESULTS

Table 1 lists the chromatographic properties of the compound formed from estriol. The infrared spectra of reference and isolated 16-epiestriol

TABLE 1
Chromatographic Properties of Isolated and Reference 16-Epiestriol and Estriol

Type of chromatography	Isolated	Reference	Estriol
Thin-layer[a]			
15% ethanol/benzene (free)	0.45	0.45	0.35
50% ethyl acetate/methylene chloride (free)	0.37	0.37	0 18
15% isopropyl ether/benzene (acetate)	0.67	0.67	0.83
0.75% ethyl acetate/methylene chloride (acetate)	0.35	0.35	0.55
Paper[b]			
Methylene chloride/ethylene glycol	0.29	0.29	1.00
Gas-liquid			
QF-1 + L-45 column (retention time)	18 min	18 min	17 min

[a] Mobility with respect to estrone as unity.
[b] Mobility with respect to estriol as unity.

were in complete agreement (Fig. 1). The compound that was isolated has the following additional chemical properties, which correspond to

those of reference 16-epiestriol: *p*-toluenesulfonic acid reaction, Kober-Ittrich reaction, absorption in sulfuric acid, and melting point.

Thin-layer chromatographic analysis of the crystalline estriol prior to dissolution in ethanol indicated that pure estriol constituted about 99% of the steroid provided by Steraloids, Inc., and Mann Research Laboratories. Less than 1% was in the form of 16-epiestriol. Once a solution was made, the quantity of 16-epiestriol increased while that of

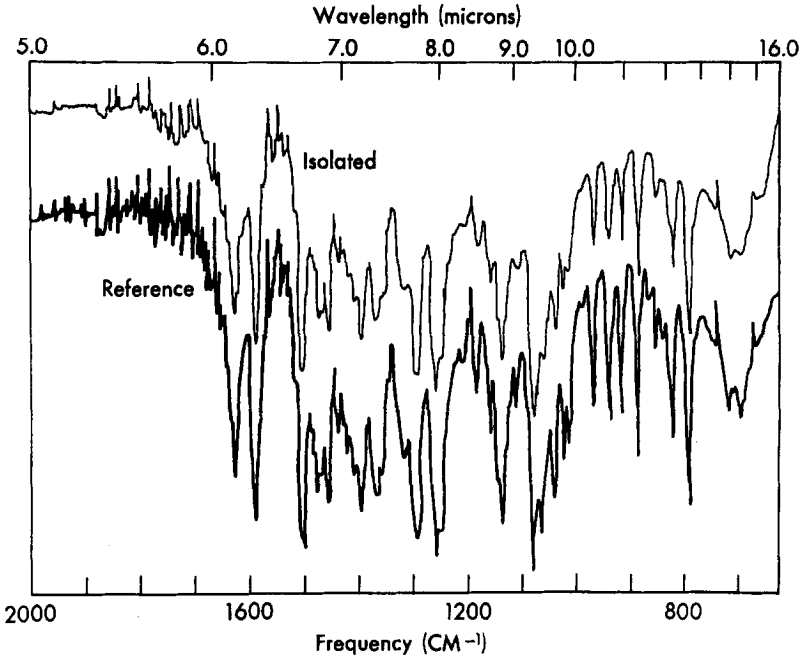

FIG. 1. Infrared absorption spectra of about 100 μg of isolated and reference 16-epiestriol in KBr pellets.

estriol decreased. This reaction proceeded rapidly at room temperature so that, after four weeks, about 10% of the estriol in solution was in the form of 16-epiestriol and, within three months, about 95% was in this form. By contrast, the material in the refrigerator changed more slowly, but within six months about 95% of this steroid had epimerized.

Estriol kept in redistilled tertiary butanol (Merck), 1 mg/ml, and frozen when not in use showed little or no epimerization.

DISCUSSION

Epimerization of estriol to 16-epiestriol without catalysis would seem unlikely from a consideration of conformational analysis. Epimerization due to the effects of ultraviolet light must be considered, although the

transformation has been seen in samples kept in the refrigerator. Impurities present in ethanol, particularly aldehydes, could be a factor. The causes of these changes are under investigation.

The results presented in this report suggest that estriol reference standards are not always stable in conditions frequently employed in the laboratory. It has been noted that estriol in biological fluids may have formed 16-epiestriol prior to analysis, as well as during analytical manipulation of nonradioactive and radioactive estriol (5). Observations of the presence of 16-epiestriol in biological fluids therefore should be re-evaluated.

SUMMARY

Estriol in ethanol solutions has been found to form 16-epiestriol. This phenomenon should be considered during the analysis of these compounds.

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