

## Estradiol 17 $\beta$ -hemisuccinate: an improved procedure

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**Summary** A simple, rapid, high-yield, and relatively inexpensive procedure for the preparation of estradiol 17 $\beta$ -hemisuccinate is described. The synthesis can be done conveniently in the ordinary biological laboratory.

**Supplementary key words** sterol esters · hapten · radioimmunoassay of estrogens

THE DEMONSTRATION BY Beiser et al. (1) that steroid protein conjugates can be used to produce antibodies that are relatively specific for the steroid hapten, and the later development of the radioimmunoassay, provided a powerful approach to the study of the steroid hormones. The development of radioimmunoassays for estrogens prepares the way for an expanded research effort concerning the physiological and pathological roles of estradiol. It is of considerable interest also in clinical diagnosis and pharmacological investigations (2).

The most commonly used hapten for the preparation of anti-estradiol antibodies is estradiol 17 $\beta$ -hemisuccinate. A literature survey did not disclose a convenient, specific method for its preparation (3). However, on a small scale the compound is commonly prepared by treating estradiol with a large excess of succinic anhydride in pyridine for 1–2 wk, isolating the resulting 3,17-disuccinate, and then hydrolyzing the phenolic ester with bicarbonate or carbonate in aqueous methanol (4) to get the desired product in fair yield. Dr. Paula Zimmering of Columbia kindly supplied us with such a procedure, and this served as a useful reference point for the synthesis

described below. The synthesis was undertaken for our own work but with the convenience and need of other biological workers in this field in mind. This preparation is simple, rapid, and requires no special skills in organic synthesis. About 4 hr of actual working time is needed, and the cost is reasonable. No attempt was made to work out optimal conditions for the reaction.

**Procedure.** 4 g (14.7 mmoles)<sup>1</sup> of  $\beta$ -estradiol (Sigma Chemical Co.) was placed in a 500-ml round-bottom flask which was fitted with a water trap (distilling trap or moisture test receiver) surmounted by a condenser and drying tube. 200 ml of benzene containing 1% pyridine (catalyst) was added to the flask, and the solution was refluxed for about 3 hr to remove water.<sup>2</sup> Next, 12 g (an excess) of succinic anhydride (Aldrich Chemical Co.) was added and refluxing was continued for about 24 hr, when estradiol was no longer detectable by TLC.<sup>3</sup> On cooling to room temperature, 6.5 g of succinic anhydride came out of solution and was filtered off.<sup>4</sup> The solvent was removed on a flash evaporator, and the residue was dissolved in about 500 ml of methanol and stirred overnight with an excess of NaHCO<sub>3</sub> (12 g suspended in 100 ml of water) to selectively hydrolyze the 3,17-disuccinate to the 17-hemisuccinate of estradiol. Completeness of the reaction was checked by TLC. After filtration an equal volume of water was added, and the alkaline solution was extracted three times with 200-ml portions of diethyl ether. The aqueous phase was brought to pH 7 with N HCl and then poured into 2 l of a mixture of 0.1 N HCl

<sup>1</sup> The reaction can be scaled up without any difficulty.

<sup>2</sup> The water trap and preliminary 3-hr reflux may be omitted if reagent grade solvents from unopened bottles are employed.

<sup>3</sup> TLC: CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH 4:1 on silica gel F-254 (EM Laboratories). The spots are visualized by UV light and by spraying with 70% perchloric acid followed by warming. *R<sub>F</sub>* values vary with sample size. Relative *R<sub>F</sub>* values are: estradiol, 1.0; estradiol 17-hemisuccinate, 0.87; estradiol 3,17-disuccinate, 0.70 (the disuccinate is partially hydrolyzed by silica gel and always gives an additional spot corresponding to the monosuccinate).

<sup>4</sup> Removal of excess succinic anhydride in this manner is not essential to the success of the preparation; it will be hydrolyzed later by NaHCO<sub>3</sub> and remain in H<sub>2</sub>O solution.

Abbreviations: TLC, thin-layer chromatography.

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and crushed ice. The white crystalline product that separated was obtained by filtration, washed with a large volume of water, and dried. Yield, 4.9 g (89%); mp, 145–147°C.<sup>5</sup> This material is pure by TLC and is suitable for making protein conjugates. It crystallizes from boiling benzene (0.5% w/v) as shiny needles and melts at 163–164°C (uncorrected; Thomas-Hoover Apparatus). Elemental analysis (CHO; C<sub>22</sub>H<sub>28</sub>O<sub>5</sub>, mol wt 372.47) and IR and NMR spectra were consistent with the structure of estradiol 17-hemisuccinate (estra-1,3,5(10)-triene-3,17β-diol-17-hemisuccinate).

It should be noted that this process is a practical means

<sup>5</sup> Occasionally, if heating is done very slowly, we obtain two melting points, 145 and 163°C, which indicates that the product can exist in two distinct crystal forms.

for obtaining a water-soluble form of estradiol (as the salt), and it should be applicable in the preparation of a wide range of sterol esters.

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