IMPROVED PROCEDURES FOR THE PREPARATION OF 2,4-DIBROMOESTRIOL AND DIDEUTERIOESTRIOL

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ABSTRACT

An improved isolation procedure is described which permits the preparation of 2,4-dibromoestriol in nearly the theoretical yield instead of the very small quantities recovered by following previously published procedures. Dehalogenation with deuterium over palladium in sodium methylate as catalyst gave deuterioestriol with 7.73 atom per cent excess deuterium (theory for dideuterioestriol, 8.32) in 93% yield.

The applicability of deuterium-labeled steroids in the estimation of estrogen production rates during normal pregnancy by the isotope dilution technique has been demonstrated by Pinkus, et al. (1). Deuterated estriol should be especially useful in similar measurements in patients with complications of pregnancy, but its preparation by application of methods described previously was entirely unsatisfactory, consistently yielding less than 5% product in a very impure mixture. The procedural modifications described here give excellent chemical yields with nearly maximum labeling, and will be of immediate practical utility to investigators in this area.

Deuterium-labeled estradiol-17β was prepared by Pinkus, et al. (1) and by Fishman, et al. (2) using the method of Coombs and Roderick for the preparation of tritiated estrogens via the brominated compounds (3). Coombs and Roderick also claimed the selective preparation of 2,4-dibromoestriol,

but we were unable to reproduce this result with their suggested procedure. When due attention was paid to the solubility of the product according to the modified procedure of isolation given below, 2,4-dibromoestriol was obtained in 90-95% yield.

A modification of Coombs and Roderick's method of tritiation employing sodium methylate in place of potassium hydroxide was shown to be applicable to the deuteration of 2,4-dibromoestriol. Specificity of labeling in the product was investigated by rebromination of the purified deuterioestriol. Mass spectrometry of the rebrominated compound showed a loss of 85% of the deuterium originally introduced which showed that less than 15% of the deuterium had entered positions other than 2 and 4 under the catalytic conditions used. Analysis by mass spectrometry of the deuterioestriol gave an atom per cent excess of 7.73 (the theoretical maximum for introduction of two deuterium atoms per estriol molecule is 8.32 atom per cent excess).

EXPERIMENTAL SECTION(4)

2,4-dibromoestriol[2,4-dibromoestra-1,3,5(10)-triene-3,16α,17β-triol]. Estriol (0.51 gm) in absolute ethanol (150 ml) was stirred at room temperature while N-bromosuccinimide (0.86 gm) was added during one hour. After stirring 18 hours more, the solvent was removed in vacuo at room temperature and the residue was dissolved in chloroform (125 ml). Water (20 ml) was added and mixed, whereupon the product precipitated in the aqueous phase which was separated. The addition of water was repeated and the combined aqueous phases were filtered to yield 0.73 gm (92%) of chromatographically homogeneous material (TLC on Eastman Chromogram sheets, 100μ silica gel; chloroform : ethanol, 9:1). This product was purified further by recrystallization from methanol. M.p. 276-277 (decomp.) (Lit[3]. m.p. 281-282°); λ_{max} 292 (shoulder 287) nm, (ε2911); ν_{max} (KBr) 3560, 2925, 1465, 1395, 1345-1335, 1193, 1058, 1027, 765 cm^{-1}; mass spectrum 448 (48), 446 (100), 444 (52) (M/e (relative intensity)).

Deuterioestriol. Absolute methanol (20 ml) was added to clean sodium metal (233 mg) under a flowing atmosphere of purified dry nitrogen and was allowed to react to completeness. The resulting solution was heated to a gentle boil with continued nitrogen flushing and 30 ml more absolute methanol were added. Then 2,4-dibromoestriol (250 mg) and 10% palladium on charcoal

(260 mg) were added and the reaction mixture was stirred briskly under deuterium gas (99.5%) for 5 hr at room temperature. The catalyst was removed by filtration and the solution was diluted with 5% acetic acid (12 ml). The mixture was evaporated in vacuo at 40° to a residue which was dissolved in 2:1 chloroform:methanol (5 ml) and then diluted with 150 ml additional chloroform. Water (30 ml) was added and mixed and the product precipitated in the aqueous phase which was separated. The addition of water was repeated and the combined aqueous phases were filtered to give 151 mg (93% yield) of chromatographically homogeneous material which migrated identically with non-labeled estriol (TLC on Eastman Chromagram sheets, 100μ silica gel; chloroform:ethanol, 9:1). Further purification was done by crystallization from ethanol. M.p. 279.5-281.5°; λ_{max} 280 (shoulder 285) nm, (ε2072); ν_{max} 3510, 3450, 2940, 1475-1455, 1424, 1255, 1277, 1075-1065, 1036 cm^{-1}.

Treatment of 300-500 μg of the product with 200 μl N, O-bis-(trimethylsilyl)-acetamide and 100 μl of trimethylchlorosilane at 60° for 4 hours gave the trimethylsilyl derivative. Direct use was made of this solution for gas chromatographic-mass spectrometric analysis; (M/e (relative intensity)), M^+ 506 (100), 416 (22), 388 (33), 362 (17), 347 (44), 326 (24), 313 (46), 299 (32), 285 (17), 272 (21), 257 (9), 247 (6), 219 (11), 169 (6). These peaks correspond to approximately 20% monodeuterioestriol, 65% dideuterioestriol, 10% trideuterioestriol, and 2% of estriol molecules with four or more atoms of deuterium per molecule of steroid. Rebromination of this product exactly as described above and analysis of the isotope distribution showed that 85% of the deuterium was displaced by resynthesis of the 2,4-dibromoestriol.

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