DETERMINATION OF ESTROGENS BY RADIOIMMUNOASSAY WITH ANTIBODIES TO ESTROGEN-C6-CONJUGATES

II. SYNTHESIS OF ESTRADIOL-17α-6-ALBUMIN CONJUGATE

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ABSTRACT

The 6-carboxymethoxime of 6-oxo-estradiol-17α was prepared and conjugated to bovine serum albumin by the mixed-anhydride-technique. The product and the intermediate were characterized by the usual methods.

INTRODUCTION

The properties of anti-estradiol-17ß-6-conjugate antiserum (1) and of anti-estriol-6-conjugate antiserum (2) were similar with regard to their low affinity for estradiol-17α, 17-epi-estriol, and 16,17-epi-estriol, and their relative high affinity for 16-epi-estriol. Obviously the extent of cross reactivity depends on the α or ß position of the particular ring D substituent which is responsible for the structural difference between the cross-reacting hapten and the hapten determinant. Assuming that the influence of this substituent on cross reactivity is more or less independent of the steric arrangement of the other substituents (these being the same in both the cross-reacting hapten and the hapten determinant), it may be speculated that cross reactivity indicates whether selectively the α or ß, or material was extracted with ethyl acetate (3 x 300 ml), the combined extracts were washed with water (2 x 75 ml) and evaporated. 1,8 g of crude 6-oxoestradiol-17α was obtained (60%) and was refluxed 1 hr with 48 ml ethanol, 5,3 ml water, 2,7 g sodium acetate and 1,7 g carboxymethoxylamine hydrochloride. After cooling 320 ml ether was added and the organic layer was extracted with 5% solution of Na_2CO_3 saturated with sodium bicarbonate (2 x 50 ml). The combined extracts were acidified with concentrated HCL. The solid obtained after filtration was crystallized from acetic acid (diluted 1 + 1 with water), from ethanol/cyclohexane 1 : 1, and from acetone; m.p. 263 - 265°C, decomposition with gas evolution. Uv: maxima at 261 nm and 310 nm (in ethanol); Mass spectrum: m/e 359 (M^+). Chromatography: Rf-values (approx., systems 1,2, and 3, as described (3)): αE_2 0,3; 0,02; 0,73; αE_2-6-oxo 0,15; 0,15; 0,45; αE_2-6-cmo 0,09; 0,45; ---;. Nmr (in D_5-pyridine; internal standard tetramethylsilane) chemical shifts in ppm:

	$C_{(18)}\underline{H}_3$	$C_{(7)}\underline{H}_1$ eq.	$C_{(17)}\underline{H}_1$-OH	$=N-O-C\underline{H}_2-COOH$
αE_2-6-cmo	0,62	3,47	3,95*	5,09

* doublet, J = 6,0

Estradiol-17α-6-albumin conjugate

The estradiol-17α-6-carboxymethoxime was coupled to bovine serum albumin by means of the mixed-anhydride technique following the procedure which ERLANGER et al. (4) used to prepare estrone-17-albumin conjugate. After purification we found 31 6-oxo-estradiol residues covalently bound to 1 molecule albumin, determined as described previously (3).

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both sides of the C6 bound steroid antigen are recognized by the antigen-sensitive cells. To check this further we are now investigating the binding characteristics of anti-estrone- and of anti-estradiol-17α-antibodies obtained by immunizing rabbits with the particular estrogen-6-albumin conjugate. These antibodies, which will be characterized in the forthcoming paper may also be valuable for estrogen assays, e.g. for the determination of estradiol-17α in blood of cattle and rabbits.

The present report describes the synthesis of the estradiol-17α-6-conjugate which was performed in analogy to the synthesis of the estrone-, estradiol-17β-, and estriol-6-albumin conjugates published previously (3).

MATERIAL

Estradiol-17α [1,3,5(10)-estratriene-3-17α-diol] (αE_2) was purchased from Sigma, Chem. Comp., St. Louis, USA; carboxymethoxyl-amine-hydrochloride (94%) from EGA-Chemie KG, Steinheim, Germany; isobutyl-chlorcarbonate from SCHUCHARDT, München, Germany; bovine serum albumin "trocken, reinst" from Behringwerke AG, Marburg, Germany and other analytical grade reagents from E. Merck, Darmstadt, Germany.

METHODS

6-Oxoestradiol-17α 6-carboxymethoxime (αE_2-6-cmo)

5 g estradiol-17α was acetylated with acetanhydride and pyridine as usual. The acetate was oxydized with CrO_3, as described (3). 3 g of the crude 6-oxoestradiol-17α-diacetate was obtained which was hydrolysed during 24 hr in 60 ml 20% (w/v) KOH in methanol at room temperature in an atmosphere of N_2. Water was added to a total of 1 l. The main part of the hydroxide was neutralized with concentrated HCL to phenolphthalein, neutralizing was finished with solid CO_2 . The organic

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