RADIOIMMUNOASSAY OF CONTRACEPTIVE STEROIDS. I. SYNTHESIS OF 6-OXOMESTRANOL 6-(O-CARBOXYMETHYL) OXIME

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ABSTRACT

A synthesis of 6-(O-carboxymethyl) oxime of 6-oxomestranol (3-methoxy-17-ethinyl-17β-hydroxy-1,3,5 (10)-estratrien-6-one) which was required for coupling with bovine serum albumin in order to produce a specific antisera for mestranol (3-methoxy-17-ethinyl-1,3,5 (10)-estratrien-17β-ol) has been described. 6-Oxoestradiol-17β 3-methyl ether was prepared from estradiol-17β 3,17-diacetate by chromic acid oxidation, followed by hydrolysis and methylation. It was converted to its O-carboxymethyloxime derivative which was smoothly oxidized by Jones reagent to the corresponding estrone derivative. This was easily ethinylated with lithium acetylide-ethylenediamine complex to the desired compound. In an alternate approach to the desired compound, it was found that 6-oxoestradiol-17β 3- methyl ether could not be converted to its ketal under any of a variety of conditions. Ethinylation of 6-oxoestrone 3-methyl ether with limited amount of lithium acetylide reagent probably gave the 17α-ethinyl derivative as was indicated from IR and UV spectra, but its identity could not be further confirmed due to its extremely poor yield.

Steroids may be rendered antigenic by attaching them covalently to a macromolecule. Antibodies to such steroid conjugates have proved valuable in radioimmunoassay (RIA) procedures (2) and in investigation of

hormone action (3). The first generationof conjugates was effected through existing functional groups, e.g., hydroxyl or carbonyl of the steroid by forming either a hemisuccinate or a O-carboxymethyloxime derivative. Antibody specificity was dependent on the position of conjugation, and it has been suggested that conjugates prepared through a position remote from structurally unique regions would enable these functional groups to be more easily recognized, thus leading to the production of more specific antisera. Recently several workers have applied this concept to the RIA of natural estrogens. By coupling bovine serum albumin to the 6-(O-carboxymethyl) oxime derivatives of natural 6-oxoestrogens (4, 5,6), they obtained more specific antisera than formerly.

In order to develop a radioimmunoassay of mestranol, which is extensively used as an antifertility agent, it is necessary to conjugate mestranol to a macromolecule. The key intermediate for this synthesis is 6-oxoestradiol-17β 3-methyl ether (I) which may be approached by two routes; (i) from estradiol-17β 3-methyl ether 17-acetate by chromic acid oxidation or (ii) from estradiol-17β 3, 17-diacetate by chromic acid oxidation followed by hydrolysis and preferential methylation. Unfortunately,

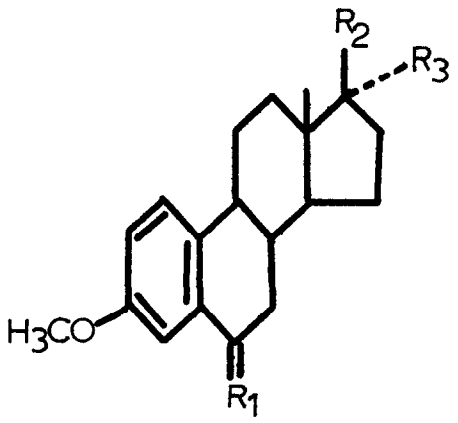
we could not oxidize estradiol-17β 3-methyl ether 17-acetate in better than 5% yield to the corresponding 6-oxo derivative whereas estradiol-17β 3, 17-diacetate on oxidation readily produced a 25-30% yield of 6-oxo-estradiol-17β 3, 17-diacetate.

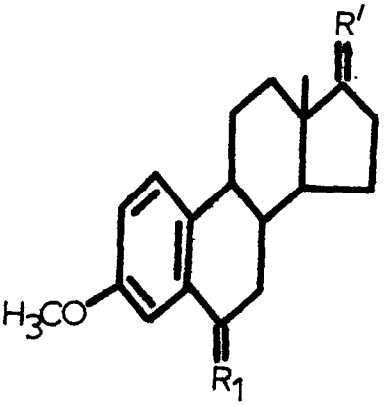
Originally we wished to protect the 6-oxo function of I by ketalization followed by Sarett's oxidation (chromic acid in pyridine) and subsequent ethinylation with lithium acetylide-ethylene diamine complex in dry dimethylsulfoxide (7). After deketalization, 6-oxomestranol could easily be converted to the corresponding oxime (IV). Unfortunately, several approaches to ketalization were unsuccessful, namely, (i) heating in benzene with ethylene glycol and p-toluene sulfonic acid removing water azeotropically, (ii) heating at 80-90° with ethylene glycol and p-toluene sulfonic acid under vacuum (7), and (iii) exchange ketal formation with butanone ethyleneketal (2-methyl-2-ethyl-1, 3-dioxolane) (8). In each case unreacted material was recovered in high yield. The unreactive nature of the 6-keto function towards ketalization may be attributed to the fact that under our experimental conditions, enol formation is

stabilized due to extended conjugation with the aromatic nucleus, thus preventing formation of the ketal.

Due to the unreactive nature of the 6-oxo compound, we thought that the corresponding estrone derivative (V) might react with a limited amount of lithium acetylide complex to produce the desired 6-oxomestranol. A trace amount of crystalline material was obtained from the reaction mixture after chromatography. While IR and UV spectra indicated the desired compound, its identity could not be further confirmed due to its extremely poor yield.

Even though 6-oxomestranol could not be prepared in satisfactory yield, the corresponding 6-(O-carboxymethyl) oxime was prepared in good yield. I was first converted to the corresponding oxime derivative (II) which was smoothly oxidized by Jones reagent (9) to produce the 17-oxo-compound (III). This compound in turn was converted to the 6-(O-carboxymethyl) oxime of 6-oxomestranol (IV) with an excess of lithium acetylide complex. The overall yield of the product from I was quite satisfactory (75-80%). This mestranol derivative was easily coupled to bovine serum albumin to produce the desired conjugate.





(I) R_1=O; R_2=OH; R_3=H (III) R_1=N-O-CH_2COOH; R'=O

(II) R_1=N-O-CH_2COOH; R_2=OH; R_3=H (V) R_1=R'=O

(IV) R_1=N-O-CH_2COOH; R_2=OH; R_3=C≡CH

EXPERIMENTAL

Oxidation of estradiol -17β 3 methyl ether 17-acetate with chromic acid in glacial acetic acid: To a solution of the compound (2.2 g) in glacial acetic acid (8 ml) at 20-22°, chromic acid (1.9 gms) dissolved in water (1.5 ml) and glacial acetic acid (12 ml) was added dropwise. After addition, the mixture was stirred for six hours at room temperature. It was diluted with water and extracted with ether. The ethereal solution, after removal of acidic byproducts by extraction with a carbonate-bicarbonate mixture, yielded an oily, neutral product (1.0 gm) which was chromatographed on silica gel. The first fraction (100 ml) of the eluent, ethyl acetate in hexane (1:4), gave a little starting material and the next 100 ml of the same eluent gave an oily product which was

crystallized from methanol, yield 120 mg (~5%), m.p. 168-69°; lit. m.p. of the corresponding 6-oxo derivative 168-70° (10). The compound was hydrolyzed with methanolic potassium hydroxide solution at room temperature to yield I, m.p. 82-84° solidifying and remelting at 130-32°; lit. m.p. 81° then 132-35° (11), 129-30° (12).

Oxidation of estradiol-17β 3, 17-diacetate with chromic acid in glacial acetic acid: This was done similarly as described by Dean et al. (4). Estradiol-17β 3, 17-diacetate (2.2 g) on oxidation yielded 550 mg (27%) of pure 6-oxoestradiol-17β 3, 17-diacetate, m.p. 174-175°; lit. m.p. 173.5-175° (4). The corresponding estradiol derivative was obtained by hydrolyzing at room temperature with methanolic potassium hydroxide solution (4%), m.p. 282-83°; lit. m.p. 280-82° (4).

Methylation of 6-oxoestradiol-17β with dimethyl sulfate and alkali: Following the procedure described by Danielli, et al. (13,) 6-oxoestradiol-17β (200 mg) was dissolved in 1 N aqueous potassium hydroxide (10 ml) giving a yellow solution. Dimethyl sulfate in 0.1 ml aliquots was added at 0° with constant shaking until a total of 1.4 ml was added. During the progress of the reaction, the color of the solution decreased and a colorless precipitate appeared. At the end, the mixture was colorless and alkaline. The precipitate was filtered, washed with water and crystallized with 50% aqueous methanol, m.p. 82-84°, solidying and remelting at 130-32°; mixed m.p. of the previous compound the same; yield 200 mg.

Preparation of 6-oxoestrone 3-methyl ether (V) from (I) with Jones reagent: 6-Oxoestradiol-17β 3-methyl ether (900 mg) was dissolved in acetone (50 ml, distilled over potassium permanganate), and the solution was cooled in ice-water. To the cold solution with stirring was added Jones reagent (0.75 ml) dropwise. After addition, it was stirred for ten minutes when a green flocculent precipitate formed. Anhydrous potassium carbonate (1 g) was added, and the mixture was stirred for another five minutes. After removal of inorganic materials by filtration, the clear filtrate was evaporated to a small volume. The material was crystallized from dilute

acetone and recrystallized from the same solvent, yield 750 mg, m.p. 145-46° (lit. m.p. 144-46°); ν_{max} 1740 (C-17 carbonyl), 1670 (C-6 carbonyl), 1605, 1570 and 1495 cm^{-1} (phenyl) in nujol.

Attempt to prepare 6-oxomestranol from V: 6-Oxoestrone 3-methyl ether (V) (300 mg) was dissolved in dry dimethylsulfoxide (20 ml). Lithium acetylide-ethylene diamine complex (150 mg, 1.5 eq) was added with stirring in a nitrogen atmosphere. Stirring was continued for 24 hours at room temperature. The deep red solution, diluted with water, was extracted thoroughly with ether. The combined ethereal extract was washed with water and dried ($NaSO_4$). The red colored oil obtained upon removing the solvent was chromatographed over silica gel (8 g). Elution was started with benzene, collecting 20 ml fractions. The first eight fractions gave some crystalline starting material; fractions 9-19 yielded a mixture of starting material and a more polar material (detected by TLC).

Fractions 20-30 yielded a small amount (70 mg) of a pinkish colored, more polar solid material, m.p. 268-72°. IR spectrum indicated a hydroxyl peak (3350 cm^{-1}) and one carbonyl peak (1710 cm^{-1}) and UV spectrum showed two peaks at 252 nm and 325 nm indicating a C-6 carbonyl group. Further characterization could not be made due to the limited amount of material.

Preparation of 6-oxoestradiol-17β 3-methyl ether 6-(O-carboxymethyl) oxime (II): II was prepared following the procedure described by Dean, et al. (4). A mixture of 6-oxoestradiol-17β 3-methyl ether (350 mg) in 80% methanol in water (42 ml), carboxymethoxylamine hemihydrochloride (350 mg) and 1 M sodium acetate (14 ml) was kept overnight at room temperature. Most of the alcohol was removed under vacuum at 40-45°, and the pH of the solution was adjusted to 4. The mixture was then extracted with ethyl acetate. The organic phase was extracted with 5% sodium carbonate in saturated sodium bicarbonate solution. Upon acidification of the cold, alkaline solution with 6 N hydrochloric acid, a colorless precipitate was obtained. It was filtered, washed, dried and recrystallized from dilute methanol: yield 350 mg, m.p. 119-22°.

ν_{max} 3400 (braod, OH), 1740 (broad, COOH), 1620, 1590, 1570 cm^{-1} (phenyl) in nujol, $\lambda_{max}^{ethanol}$ at 258 nm (ε = 10,300), at 308 nm (ε = 3,800).

Found: C, 65.4; H, 7.1; N, 3.5

Calcd. for: $C_{21}H_{27}O_5N$, 1/2 H_2O C, 65.8; H, 7.3; N, 3.6

Preparation of 6-oxoestrone 3-methyl ether 6-(O-carboxymethyl)oxime (III): II (300 mg) dissolved in acetone (25 ml) (distilled over potassium permanganate) was oxidized with Jones reagent (0.2 ml) and worked up as described before. It was crystallized from dilute methanol; yield 230 mg, m.p. 197-98° d. ν_{max} 3200 broad OH), 1745 (C-17 CO), 1730 (COOH), 1615, 1580, 1490 cm^{-1} (phenyl) in nujol. $\lambda_{max}^{ethanol}$ at 258 nm (ε = 8,800) and at 308 nm (ε = 3,300).

Found: C, 67.6; H, 7.1; N, 3.8

Calcd for: $C_{21}H_{25}O_5N$, C, 67.9; H, 6.7; N, 3.8

Preparation of 6-oxomestranol 6-(O-carboxymethyl) oxime (IV): To a solution of lithium acetylide-ethylene-diamine complex (1.5 g) in dry dimethylsulfoxide (15 ml) was added a solution of III (744 mg) in dry dimethylsulf-oxide (10 ml) with stirring in a nitrogen atmosphere at room temperature. After stirring at room temperature for six hours, the brown solution was poured into ice-water. Upon acidification with 6 N hydrochloric acid, a sandy colored precipitate was obtained (670 mg) which could not be purified by crystallization. Further purification was accomplished by extracting an ethyl acetate solution with 5% sodium carbonate in saturated bicarbonate solution and acidifying the alkaline solution with 6 N hydrochloric acid. A whiter solid, m.p. 120-28° was obtained, but we were unable to crystallize it. It was dissolved in 1:1 ethyl acetate:benzene and passed through a column containing silica gel washed thoroughly with the same solvent. A colorless semi-solid mass was obtained after evaporation of the solvent which did crystallize from a mixture of ether and hexane; yield 650 mg, m.p. 125-28°, ν_{max} 3400 (OH), 3290 (ethinyl),

2110 (ethinyl, small), 1740 (COOH), 1620, 1590, 1490 cm^{-1} (phenyl). $\lambda_{max}^{ethanol}$ at 258 nm (ε =10,000) and at 308 nm (ε =3,700).

Found: C, 69.2; H, 7.0; N, 3.4

Calcd. for: $C_{23}H_{27}O_5N$, C, 69.5; H, 6.8 and N, 3.5.

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