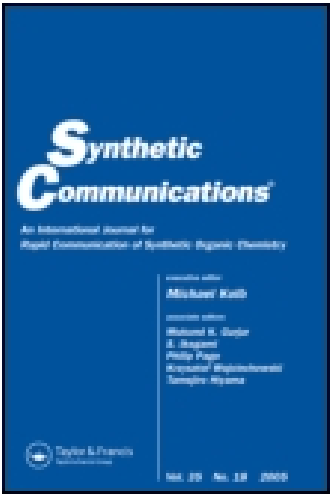

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An Efficient Synthesis of 6-Oxo-17-β-Estradiol and its O-Carboxymethyl Oxime

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AN EFFICIENT SYNTHESIS OF 6-OXO-17-β -ESTRADIOL AND ITS *O*-CARBOXYMETHYL OXIME

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Abstract: Estradiol was efficiently oxidized into 6-oxo estradiol using pyridinium chlorochromate. Previously reported yields were considerably increased by the use of oxidizing agent adsorbed onto celite. The oxo compound was then transformed into the corresponding *O*-carboxymethyl oxime derivative in quantitative yield.

A number of immunoassay systems have been developed for steroid hormones quantitation. In antibody production haptens need to be covalently attached onto a carrier protein before immunization. *O*-(Carboxymethyl) oximes, hemisuccinates, carboxyethyl ethers, and thioethers are frequently used in steroid immunoessays[1]. Highly specific antibodies exist for a number of steroids and the production of each one required the synthesis of haptens involving regiospecific chemical modifications of the parent compound. Antibodies for 17-β-estradiol have been produced using haptens obtained from estradiol modified at position 3

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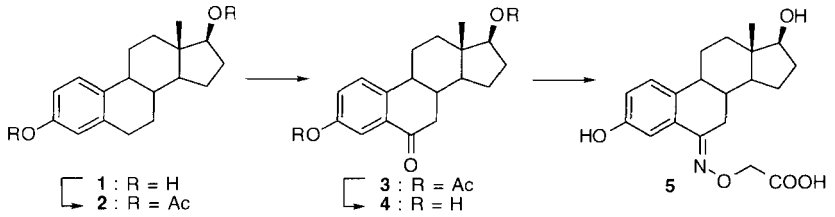
or 17[1]. However, cross-reactivity with estrone and 17-α-estradiol emerged as a serious limitation. A second generation of antibodies exhibiting enhanced selectivity and lower cross-reactions was obtained using 17-β-estradiol modified at the 6 position (scheme 1)[2].



- Scheme 1 -

17-β-Estradiol diacetate obtained from estradiol was oxidized with chromium trioxide in glacial acetic acid to afford the 6-oxo compound in 9 % yield. Other authors have reported this oxidation step using chromium oxide and 3,5-dimethyl pyrazole in dichloromethane with a slightly higher yield (18 %)[3]. The synthesis of 17-β-estradiol-6-(*O*-carboxymethyl) oxime was realized using *O*-carboxymethyl hydroxylamine hemihydrochloride and sodium acetate in aqueous methanol. Though the yield of that transformation was not clearly stated by the authors, it is assumed that it was less than 28 % with regard to the results they reported. So starting from 17-β-estradiol **1**, 17-β-estradiol-6-(*O*-carboxymethyl) oxime **2** was obtained with a 2.4 % overall yield.

In the course of our studies on new techniques for immunoessays, we were prompted to revisit the two chemical transformations mentionned above.

Herein we wish to describe new experimental procedures allowing the obtention of 17-β-estradiol-6-(*O*-carboxymethyl) oxime in 65 % yield starting from 17-β-estradiol.

Oxidation of 17-β-estradiol diacetate **2** was investigated using different oxidizing reagents (SeO_2, $Ca(ClO)_2$, CrO_3, CrO_3/*t*-BuOOH, PCC, PDC, PDC/*t*-BuOOH). Best results were obtained using pyridinium chlorochromate in refluxing benzene. Reagent agglomeration and formation of a gangue however seriously reduced the kinetics of the reaction and prolonged reaction time led to partial degradation of the product. That difficulty was removed by mixing and grinding PCC with celite (1/2.5, w/w) before use. That procedure allowed to reduce reaction time and 6-oxo-17-β-estradiol diacetate **3** could be obtained in 65 % yield. The later compound was quantitatively deacetylated using 20 % potassium hydroxide in aqueous methanol to yield 6-oxo-17-β-estradiol **4**.

Reaction of 6-oxo-17-β-estradiol **4** with *O*-carboxymethyl hydroxylamine hemihydrochloride using the procedure established by Dean *et Al.* (aqueous methanol, 0.4 M AcONa, room temperature, 16 h) afforded 17-β-estradiol-6-(*O*-carboxymethyl) oxime **5** only in modest yields (30 %)[2]. We have worked out a convenient procedure allowing the quantitative transformation of 6-oxo-17-β-estradiol diacetate **3** into oxime **5**. Oxo-compound **3** was allowed to react with *O*-carboxymethyl hydroxylamine hemihydrochloride in anhydrous methanol at

room temperature for 22 h, without any addition of sodium acetate. 17-β-estradiol-6-(*O*-carboxymethyl) oxime diacetate was obtained as its methyl ester and partial removal of the two acetates occured during the reaction. The crude reaction mixture was treated in aqueous methanol with potassium carbonate without intermediate purification and quantitatively afforded compound **5**. 6-Oxo-17-β-estradiol **4** similarly reacted with *O*-carboxymethyl hydroxylamine hemihydrochloride. In that case too, the *O*-carboxymethyl oxime was obtained in 100 % yield as its methyl ester that could be conveniently purified on silica gel and further quantitatively hydrolyzed to the corresponding acid **5** using lithium hydroxide in aqueous methanol.

Experimental[5]

6-Oxo-17-β-estradiol diacetate (**3**): 17-β-estradiol diacetate[6] (0.356 g, 1.0 mmol) was stirred in refluxing benzene and a mixture of PCC (2.0 g, 9.2 mmol) and celite (4.6 g) finely powdered in a mortar was added by portions over a 6 h period. The reaction mixture was filtered and the filtrate was reduced *in vacuo*. The crude residue was purified by chromatography on silica gel to yield non-reacted 17-β-estradiol diacetate (0.046 g) and compound **3** (0.209 g, 65 %). Analytical data were consistent with those described in the literature[3]. Additional data: ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 197.0; 171.3; 169.6; 149.4; 144.5; 133.8; 127.1;

126.9; 120.2; 82.3; 49.9; 44.0; 43.1; 42.9; 39.6; 36.6; 27.5; 25.5; 23.1; 21.3; 21.2; 12.0.

17-β-Estradiol-6-(*O*-carboxymethyl) oxime (**5**): 6-Oxo-17-β-estradiol diacetate (0.101 g, 0.27 mmol) was stirred overnight in anhydrous methanol with *O*-carboxymethyl hydroxylamine hemihydrochloride (0.033 g, 0.30 mmol). The resulting mixture of esterified and partially deacetylated compounds was reduced *in vacuo*, dissolved in *n*-butanol, washed with water and evaporated again. The residue was dissolved in 6 ml of methanol and 0.3 ml of water. Potassium carbonate (0.133 g, 0.96 mmol) was added and the solution was stirred overnight at room temperature. After evaporation of the reaction mixture, residual salts were removed *via n*-butanol/water extraction to yield compound **6** (0.097 g, 100 %). Analytical data were consistent with those described in the literature[2]. Additional data: ^{13}C-NMR ($CDCl_3$:CD_3OD 1:2, 75 MHz) δ 173.0; 155.8; 155.0; 134.5; 130.8; 125.8; 117.1; 110.3; 81.1; 70.5; 50.5; 43.0; 41.6; 37.2; 36.2; 29.6; 29.5; 25.6; 22.9; 10.7. IR (film): 3322; 2926; 1732; 1574; 1226; 1098; 1049; 876.

References

1. Pratt, J.J. *Clin. Chem.* **1978,** *24*, 1869.
2. Dean, P.D.G.; Exley, D.; Johnson, M.W. *Steroids* **1971,** *18*, 593.
3. Weber, G.; Schaumann, J.; Carl, C.; Schwarz, S. *J. Prakt. Chem.* **1989,** *331*, 223.
4. Pouzar, V.; Slavikova, T.; Cerny, I. *Collect. Czech. Chem. Commun.* **1996,** *61*, 404.

5. Full general statements were described elsewhere: Ducray, P., Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1996**, *79*, 2346.

6. 17-β-estradiol diacetate is quantitatively obtained from 17-β-estradiol using the procedure described in reference 2.

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