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PREPARATION OF Nε-(3-HYDROXY-16-OXOESTRA-1,3,5(10)-TRIEN-17β-YL)-L-LYSINE, 16α-HYDROXYESTRONE-LYSINE ADDUCT, *VIA* HEYNS REARRANGEMENT

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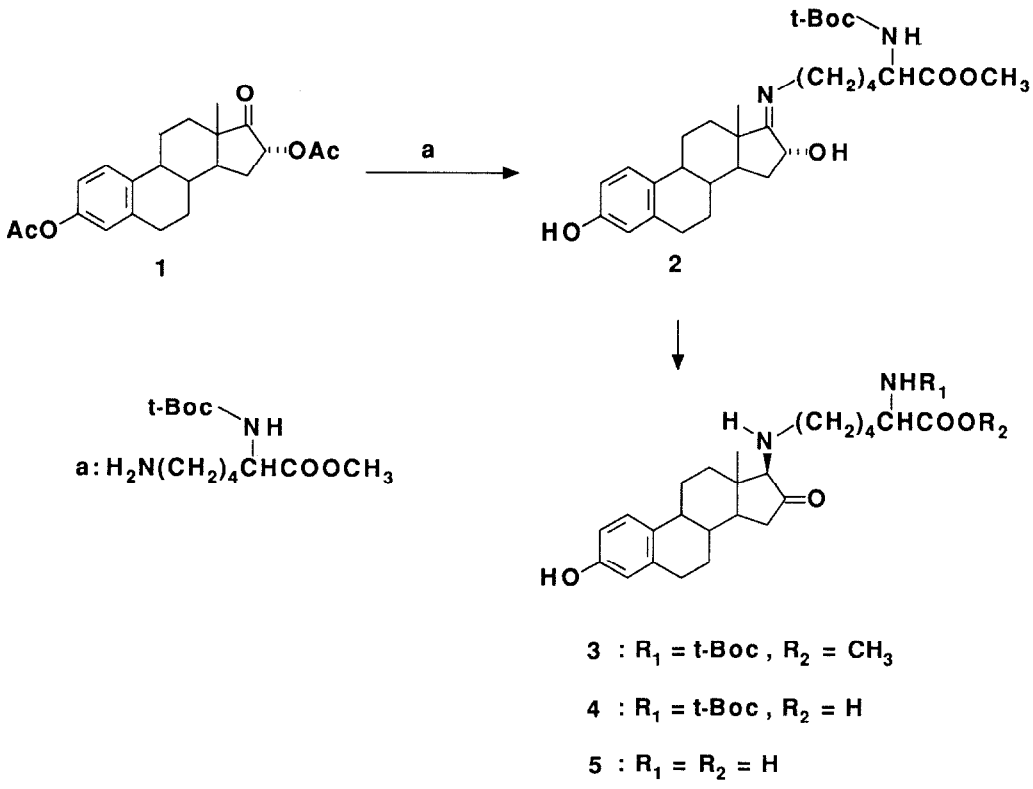
Summary: Preparation of a stable covalent adduct from 16α-hydroxyestrone with the ε-amino group of lysine via two step reactions involving Schiff base formation followed by Heyns rearrangement is described.

It has been established that 2- and 16α-hydroxylation reactions are the two major pathways in estrogen metabolism, and 2- and 16α-hydroxyestrones are the primary products by the hydroxylation enzymes in man[1]. The two estrogen metabolites have been the subject of much discussion whether one or both of them is/are implicated in the oncogenic event(s) in breast tissues. Recently, it has been demonstrated that the rate of estrogen 16α-hydroxylation increased significantly in the breast cancer patients while that of 2-hydroxylation was too variable to be significant[2]. Furthermore, it has been clarified that radio-labeled 16α-hydroxyestrone was retained stably on nuclear components such as estrogen receptor isolated from MCF-7 human breast cancer cells[3] and histone[4]. The mechanism for formation of the stable covalent adduct from 16α-hydroxyestrone with protein has been postulated *via* Heyns rearrangement following Schiff base formation at the 17-position with the lysinyl residue[5]. However, there has been reported a study using a model compound which dealt with the rearrangement of the steroidal D-ring α-hydroxyimine prepared from 16α-hydroxyestrone with 2-methoxyethylamine[6]. Therefore, it seems to be of importance to characterize a stable adduct formed from 16α-hydroxyestrone with the ε-amino group of lysine.

It is sufficiently substantiated that 16α-hydroxyestrone readily undergoes the ketol rearrangement in basic condition yielding 16-oxoestradiol. Accordingly, the 3,16-diacetate(**1**) was subjected to the reaction with Nα-t-butoxycarbonyl-L-lysine methyl ester (*ca.* 15 equivalent moles) without any solvent to form the Schiff base (**2**) at the 17-ketone. During the reaction at room temperature for 16 hrs, both acetates were hydrolyzed spontaneously. The reaction mixture was, then, brought to the 33% aqueous MeOH solution (adjusted to neutral with citric acid) to accelerate Heyns rearrangement. The product was extracted into EtOAc and purified by successive column chromatography on silica gel using benzene-EtOAc (1:2), $CHCl_3$-EtOAc (2:1) and hexane-EtOAc-$CHCl_3$ (8:8:1) as eluents. Compound 3 was thus obtained as an oil in overall yield of 27%. Usual hydrolysis of **3** with NaOH provided compound **4** in 79% yield which in turn was treated with trifluoroacetic acid in anisole under icc-cooling to remove the t-butoxycarbonyl group. The crude product was dissolved in 5% HCl and washed with $CHCl_3$. Upon neutralization the resulting precipitate was collected and combined with the extract on a Sep-Pak C_{18} cartridge from the mother liquor. The purified product was recrystallized from dil. HCl-CH_3CN-acetone to give Nε-(3-hydroxy-16-oxoestra-1,3,5(10)-trien-17β-yl)-L-lysine(**5**) as HCl salt in 57% yield[7].

The formation of the stable adduct from 16α-hydroxyestrone with the ε-amino group of lysine has proved to proceed along the two step mechanism involving Schiff base formation and Heyns rearrangement. The availability of the authentic adduct would serve for further studies on the relationship between estrogen metabolism and breast cancer.



Scheme 1

Acknowledgements

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7. mp. 153 °C (decomp). Anal. Calcd for $C_{24}H_{34}N_2O_4 \cdot 2HCl$:C,59.14;H,7.44;N,5.75. Found:C,59.43; H,7.59;N,5.61. ^{1}H-NMR(D_2O/CD_3OD(5:1))δ:0.83(3H,s,18-CH_3),3.78(1H,s,17α-H),3.96(1H,t,*J*=5.0Hz, -CHCOO),6.45-6.76(2H,2- and 4-H),7.13(1H,d,*J*=8.0Hz,1-H).

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