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19-ACETYLENIC, 19-HYDROXY-ANDROST-4-EN-3,17-DIONES. POTENTIAL SUICIDE SUBSTRATES OF ESTROGEN BIOSYNTHESIS

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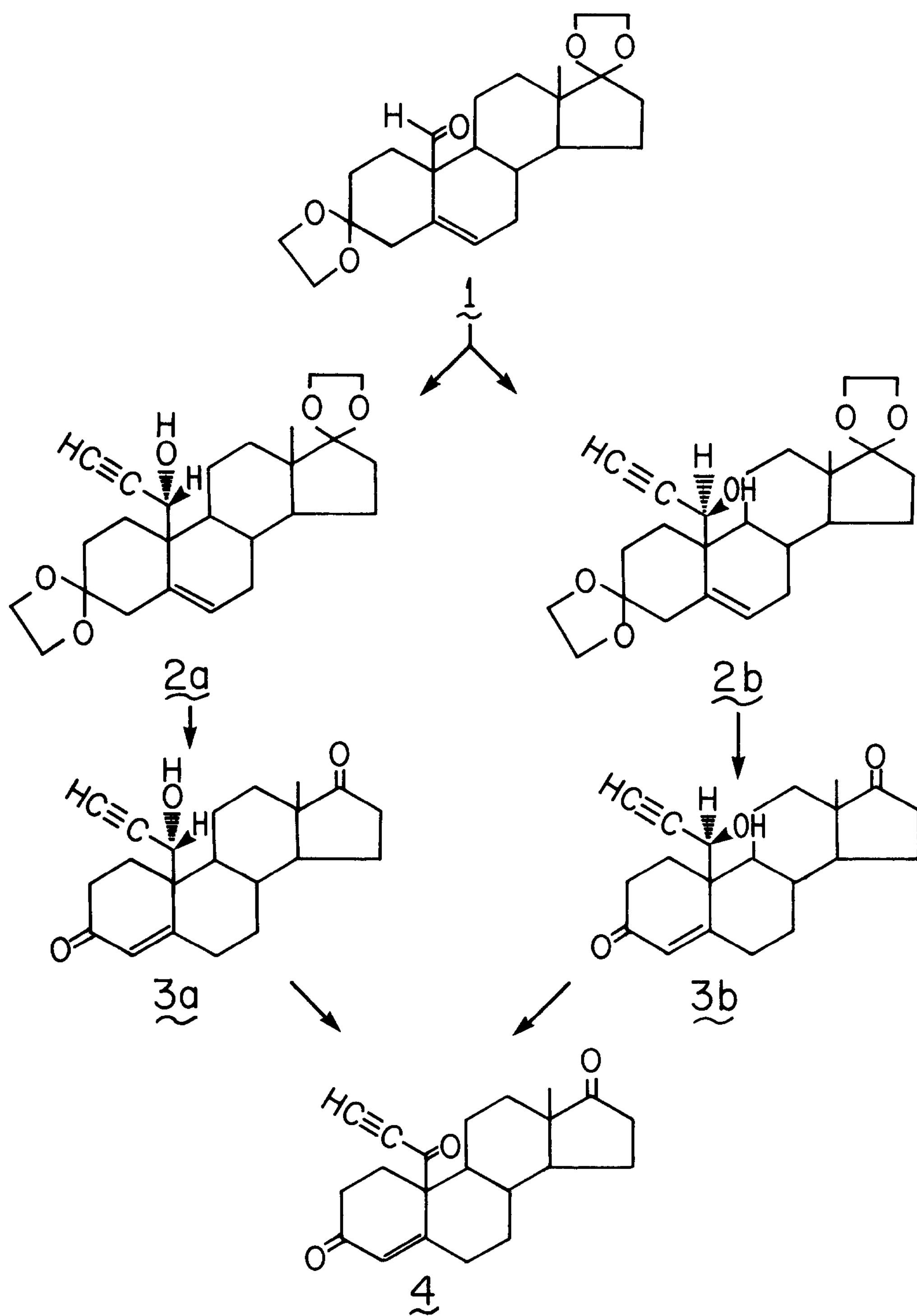
Steroids designed as potential suicide substrates of estrogen biosynthesis are reported. Enzyme inhibition resulting from covalent modification should occur if these acetylenic alcohols are converted into a reactive acetylenic ketone by the aromatase enzyme system.

Estrone and estradiol are formed enzymatically from their respective precursors, androstenedione and testosterone, by the aromatase enzyme system. It is known that 3 moles of O_2 and NADPH are consumed in the conversion.[1] There is now general concensus that the first 2 moles of O_2 and NADPH are used in sequential hydroxylations of the 19-methyl group. Dehydration of this 19-*gem* diol leads to the readily isolated 19-aldehyde intermediate.[2] Evidence for several pathways has been presented regarding the utilization of the remaining mole of O_2 and NADPH in the conversion of this aldehyde to the estrogen product.[3-5]

Inhibitors of estrogen biosynthesis would be potentially useful agents for the treatment of endocrine disorders and estrogen dependent tumors. We are investigating the possibility of designing compounds which would be suicide substrates for the aromatase enzyme system. Such compounds should be converted into reactive electrophilic species only at the enzyme's activite site when it carries out its normal catalytic event. This approach holds the promise of being able to achieve selective irreversible covalent modification of the enzyme *in vivo*. Accordingly, we have prepared the 19-acetylenic alcohols **3a** and **3b** as potential suicide substrates. Hopefully enzymatic hydroxylation at C-19, followed by *in situ* dehydration will produce the 19-acetylenic ketone **4**. Michael addition to the acetylenic ketone by an enzyme nucleophile should lead to covalent modification and subsequent loss of enzymatic activity.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Aldehyde **1** was prepared in ten steps from dehydroepiandrosterone according to the literature[6-8] and ethynylated with acetylenedimagnesium bromide in refluxing THF for 2 hrs. to give a ca 60:40 mixture of alcohols **2a**:**2b** in 94% yield. The alcohol mixture was separated by HPLC (μPorasil eluted with 15% CH_3CN, 21% $(ClCH_2)_2$, 64% hexane). Compound **2a** had[9]:mp 188-190°C; nmr 2.57 (d, J=2.5, 1, H-C≡C-), 4.83 (t, J=2.5, 1, CH-OH), 5.60 (m, 1, H-C=C-); ir 3535, 3355, 2105. Alcohol **2b** had: mp 180-182°C; nmr 2.53 (d, J=2, 1, H-C≡C-), 4.70 (d of d, J=2, J=7, CH-OH), 5.60 (m, 1, H-C=C-), ir 3540, 3350, 2115. Assuming that the addition of acetylenedimagnesium bromide to the 19-aldehyde follows the same steric course as the addition of methyl lithium to 3β,17β-diacetoxy-5-androsten-19-al one would expect **2a**, the major product, to have the S configuration at C-19[10]. The minor product **2b** should have the R configuration. *These assignments must be considered tentative at this time.*

Alcohol **2a** was converted into compound **3a** in 79% yield by stirring overnight in acetone containing 10% aqueous sulfuric acid. Alcohol **3a** had: mp 191-193°C, nmr 2.47 (d, J=2, 1, H-C≡C-), 4.83 (d of d, J=2, J=5.5, 1, CH-OH), 3.62 (d, J=5.5, 1, CH-OH), 5.75 (s, 1, H-C=C-); ir 3525, 3370, 2125, 1735, 1660, 1625; UV λ_{max} 239, ε=15,100. Alcohol **3b** was similarly prepared from **2b** in 88% yield and had: mp 180-182°C, nmr 2.70 (d, J=2, 1, H-C≡C-), 2.95 (d, J=3.5, 1, CH-OH), 4.90 (m, J=2, J=3.5, 1, CH-OH), 5.93 (br s, 1, H-C=C-); ir 3500, 3290, 2120, 1740, 1670, 1625; UV λ_{max} 238, ε=14,400.

Alcohols **3a** and **3b** were each separately oxidized in 69% and 73% yield respectively, with Jones reagent in acetone to give acetylenic ketone **4** which had: mp 167.5-169°C, nmr 3.47 (s, 1, H-C≡C-), 5.97 (br d, J=2, 1, H-C=C-); ir 3265, 2095, 1740, 1665, 1625; UV λ_{max} 216(sh), 225(sh), 243, ε=10,300, 11,700 and 13,200 respectively.

Biological evaluation of these compounds will be reported elsewhere.

Acknowledgements

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References and footnotes

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9. All compounds had correct C,H, analyses. Melting points are uncorrected. Nmr spectra were recorded in $CDCl_3$ on a Varian T-60 nmr and resonance signals reported as δ values. Coupling constants are in Hz. Ir spectra were recorded in KBr on a PE-137 instrument and reported in cm^{-1}. UV spectra were recorded in CH_3CN on a Cary 118 spectrophotometer Maxima are reported in nm and extinction coefficients in $cm^{-1}M^{-1}$.

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