THE CHEMISTRY OF 9α-HYDROXYSTEROIDS. 1. PREPARATION OF 9α,17β-DIHYDROXY-17α-ETHYNYLANDROST-4-EN-3-ONE

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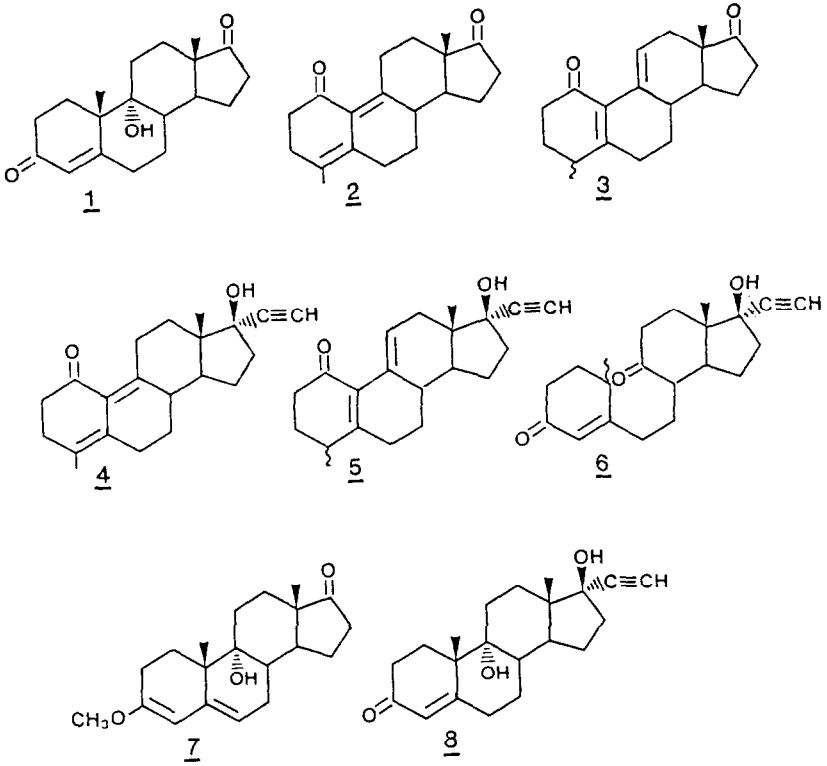
ABSTRACT

9α-Hydroxyandrost-4-ene-3,17-dione 1, when allowed to react with dipotassium acetylide in tetrahydrofuran, resulted, after chromatographic separation, in 4-methyl-19-norandrosta-4,9-diene-1,17-dione 2, 4ξ-methyl-19-norandrosta-5(10),9(11)-diene-1,17-dione 3, 4-methyl-17α-ethynyl-17β-hydroxy-19-norandrosta-4,9-dien-1-one 4, 4ξ-methyl-17α-ethynyl-17β-hydroxy-19-norandrosta-5(10),9(11)-dien-1-one 5, and 17α-ethynyl-17β-hydroxy-9,10-secoandrost-4-ene-3,9-dione 6. Selective protection of Δ^4-3-ketone of 9α-hydroxyandrost-4-ene-3,17-dione 1 as its dienol methyl ether 7, and subsequent reaction with lithium acetylide-ethylenediamine followed by acidic hydrolysis, afforded 9α,17β-dihydroxy-17α-ethynylandrost-4-en-3-one 8.

INTRODUCTION

Recent developments in the microbial degradation of sterols* have resulted in a straightforward and economically attractive process for the production of 9α-hydroxyandrost-4-ene-3,17-dione 1. This has prompted us to attempt the introduction of an ethynyl group at C-17 while keeping the C-9α-OH group intact. The 9α-hydroxyethynylsterone 8, thus produced, can serve as a new and interesting intermediate for the preparation of corticosteroids (1-3).



EXPERIMENTAL

9α-Hydroxyandrost-4-ene-3,17-dione is manufactured at Gistbrocades, 2600 MA Delft, The Netherlands.

Melting points were measured on a melting point apparatus (oil bath) and were uncorrected. UV spectra were determined on a Unicam SP 1800 spectrophotometer. Infrared spectra were recorded in KBr on a Perkin-Elmer 521 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker AM 360 MHz spectrometer. The NMR spectra were taken in $CDCl_3$, unless otherwise mentioned, and chemical shifts reported on the δ scale in parts per million downfield from Me_4Si as internal standard.

Reaction of Dipotassium Acetylide with 9α-Hydroxyandrost-4-ene-3,17-dione 1

9α-Hydroxyandrost-4-ene-3,17-dione 1 (1.495 g) in dry THF (20 mL) was added slowly to a slurry of dipotassium acetylide (prepared by passing acetylene into a solution of potassium tert-butoxide

(1.172 g) in dry THF (50 mL) kept at about -20 C) at -10 to -15 C. The reaction mixture was stirred at the same temperature for 45 min and then at 20 C for 1.15 h. Next, the reaction mixture was diluted with cold H_2O and brought to pH 4.0 with 4 N HCl. Most of the solvents were removed under reduced pressure and the contents extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure and dried under vacuum to give a thick oily liquid (yield = 1.868 g).

The crude product was chromatographed over HPLC (Merck Column A) using toluene/ethyl acetate (5:1) as the eluent. The following three compounds were isolated.

4-Methyl-19-norandrosta-4,9-diene-1,17-dione 2
mp 119-123 C (toluene/ethyl acetate); UV λmax (MeOH) 282 nm (ε 2,749); IR: 1740 (C-17=O), 1670 (C-1=O)cm^{-1}. ^{1}H-NMR: 0.999 (S, 3H, C-18H_3), 1.758 (S,3H,C-4 CH_3). ^{13}C-NMR: 13.05 (C-18), 19.05 (C-4 CH_3), 127.11, 128.90, 130.10, 143.13, (C-4,C-5,C-9,C-10), 203.66 (C-1), 215.68 (C-17). Mass spectrum: M^+ = 284.

4ξ -Methyl-19-norandrosta-5(10),9(11)-diene-1,17-dione 3
mp 140-143 C (toluene/ethyl acetate); UV λmax (MeOH) 284 nm (ε 5,675); IR: 1740 (C-17=O), 1670 (C-1=O)cm^{-1}, ^{1}H-NMR: 0.898 (S,3H,C-18H_3), 0.911 (S,3H,C-18H_3), 1.20 (d,3H,C-4 CH_3), 1.23 (d,3H,C-4 CH_3), 6.72 (br.S,1H,C-11 H), 6.89 (br.S,1H,C-11 H). ^{13}C-NMR: 14.37 (C-18), 17.56 (C-4 CH_3), 18.77 (C-4 CH_3), 124.43 (C-11), 124.66 (C-11), 129.35, 130.13 (C-5,C-10), 159.27 (C-9), 199.08 (C-1=O), 214.25 (C-17=O). Mass spectrum: M^+ = 284.

4-Methyl-17α-ethynyl-17β-hydroxy-19-norandrosta-4,9-dien-1-one 4
mp 199-205 C (decomp.) (toluene/ethyl acetate); UV λmax (MeOH) 294 (ε 3,449); IR: 3450 (OH), 3240 (≡CH), 2100 (C≡C), 1680 (C=O)cm^{-1}. ^{1}H-NMR: 0.986 (S,3H,C-18H_3), 1.745 (S,3H,C-4 CH_3), 2.55 (S,1H,≡CH). ^{13}C-NMR: 11.96 (C-18), 19.00 (C-4 CH_3), 74.11 (C-21), 79.44 (C-20), 86.97 (C-17), 126.37, 129.17, 129.59, 144.40 (C-4,C-5,C-9,C-10), 203.96 (C-1=O). Mass spectrum: M^+ = 310.

Thereafter, using toluene/ethyl acetate (2:1) as the eluent, the fractions containing 5 and 6 were collected.

4ξ-Methyl-17α-ethynyl-17β-hydroxy-19-norandrosta-5(10),9(11)-dien-1-one 5
UV λmax (MeOH) 288 nm: IR: 3420 (OH), 3290 (≡C), 1665 (C=O)cm^{-1}. ^{1}H-NMR: 0.866 (S,3H,C-18H_3), 0.883 (S,3H,C-18H_3), 1.21 (d,3H,C-4 CH_3), 1.2 (d,3H,C-4 CH_3), 2.57 (S,1H,≡CH), 6.76 (br.S,1H,C-11 H) 6.93 (br.S,1H,C-11 H). ^{13}C-NMR: 12.71(C-18),18.84 (C-19), 74.20 (C-21), 79.84 (C-20), 125.76 (C-11), 199.46 (C-1), 212.39 (C-17).

Mass spectrum: M^+=310.

17α-Ethynyl-17β-hydroxy-9,10-secoandrost-4-ene-3,9-dione 6
mp 140-145 C (toluene/ethyl acetate); UV λmax (MeOH) 238 nm (ε 4,598); IR: 3400 (OH), 3245 (≡C-H), 2090 (C≡C), 1730 (C-9=O), 1660 (C-3=O)cm^{-1}. ^{1}H-NMR: 0.963 (S,3H,C-18H_3), 0.950 (S,3H,C-18H_3), 1.21 (d,3H,C-10H_3), 2.50 (S,1H,≡CH), 5.87 (S,1H,C-4 H), ^{13}C-NMR: 13.54 (C-18), 17.76 (C-10 CH_3), 74.79 (C-21), 73.61 (C-20), 84.56 (C-17), 125.09 (C-4), 170.29 (C-5), 199.64 (C-3=O), 216.92 (C-9=O). Mass spectrum: M^+ = 328.

9α-Hydroxy-3-methoxyandrosta-3,5-dien-17-one 7
Trimethyl orthoformate (2.2 mL) was added to a stirred suspension of 9α-hydroxyandrost-4-ene-3,17-dione 1 (2.2 g) in CH_3OH (30 mL), followed by dropwise addition of a 5% solution of H_2SO_4 in CH_3OH until a pH meter showed a value of 0.4. After stirring for 1 h at room temperature, triethylamine was added until pH=7 was reached. Next, H_2O (2 mL) was added and pH was adjusted to 2.5 by addition of 1 N H_2SO_4, and the contents were further stirred for 30 min at room temperature. The reaction mixture was brought to pH=9.0 with triethylamine, diluted with H_2O (20 mL), and stirred vigorously under ice-water bath cooling for 30 min. The resulting precipitate was collected, washed with cold H_2O, and dried under vacuum to a constant weight to yield 1.3 g of 7. mp 196-201 C; UV λmax (MeOH) 240 nm (ε 19,674); IR: 3580 (OH), 1734 (C=O), 1645 (C=C), 1612 (C=C) cm^{-1}. ^{1}H-NMR: 0.905 (S,3H,C-18H_3), 1.119 (S,3H,C-19H_3), 3.55 (S,3H,OCH_3), 5.16 (S,1H,C-4 H), 5.31 (tr, 1H,C-6 H). ^{13}C-NMR: 12.64 (C-18), 21.75 (C-19), 54.16 (C-3 OCH_3), 74.16 (C-9), 98.59 (C-4), 116.58 (C-6), 138.28 (C-5), 155.38 (C-3), 215.26 (C-17). Mass spectrum: M^+ = 316.

9α,17β-Dihydroxy-17α-ethynylandrost-4-en-3-one 8
A solution of 9α-hydroxy-3-methoxyandrosta-3,5-dien-17-one 7 (1.072 g) and lithium acetylide-ethylenediamine (90%, 1.663) in dry THF (7 mL) was stirred at room temperature for 20 h. The reaction mixture was then cooled, the pH adjusted to 1.0 with 4 N HCl, and further stirred at room temperature for 45 min. Next, most of THF was evaporated under reduced pressure and the contents extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with saline and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the product dried under vacuum to yield 0.708 g of 8. This product was further purified by HPLC (Merck Column B) using toluene/ethyl acetate (2:1) as the eluent. mp 163-168 C (toluene/ethyl acetate); UV λmax (MeOH) 238 nm (ε 15,015); IR: 3616 (OH), 3400 (OH), 3266 (=CH), 2094 (C≡C), 1646 (CO), 1607 (C=C)cm^{-1}. ^{1}H-NMR (DMSO-d_6): 0.748 (S,3H,C-18 H_3), 1.230 (S,3H,C-19H_3), 3.21 (S,1H,C-21 H), 4.03 (OH), 5.23 (OH), 5.62 (S,1H,C-4 H). ^{13}C-NMR (DMSO-d_6): 12.03 (C-18), 19.56

(C-19), 75.36 (C-21), 125.01 (C-4), 75.04 (C-9), 78.06 (C-17), 89.15 (C-20), 171.03 (C-5). Mass spectrum: m/e = 328, 310, 295, 284.

RESULTS

Attempts to carry out selective addition of a two-carbon fragment to the C-17 ketone function of 9α-hydroxyandrost-4-ene-3,17-dione 1 via reaction with dipotassium acetylide (2) in dry tetrahydrofuran did not yield the desired ethynylsterone 8. Preparative HPLC of the reaction mixture allowed the isolation of the rearranged steroids 4-methyl-19-norandrosta-4,9-diene-1,17-dione 2, 4ξ-methyl-19-norandrosta-5(10),9(11)-diene-1,17-dione 3, and the corresponding C-17 hydroxyethynylated products 4 and 5. In addition to these compounds, 17α-ethynyl-17β-hydroxy-9,10-secoandrost-4-ene-3,9-dione 6 in two stereoisomeric forms (at C-10) was isolated. Bergstrom and Dodson (4) reported the formation of 3 while attempting dehydration of 1 with 4-toluenesulfonic acid in benzene.

However, under controlled reaction conditions 9α-hydroxy-androst-4-ene-3,17-dione 1 reacted with trimethyl orthoformate (5) in CH_3OH containing H_2SO_4 to give crystalline 9α-hydroxy-3-methoxyandrosta-3,5-dien-17-one 7. Reaction of lithium acetylide-ethylenediamine with the $\Delta^{3,5}$-dienol methyl ether 7 in dry THF followed by deprotection with 4 N HCl provided 9α,17β-dihydroxy-17α-ethynylandrost-4-en-3-one 8 as the target compound, which was characterized by IR, ^{13}C-NMR, and ^{1}H-NMR spectra. Mass spectro-

metric analysis showed molecular ion at m/e 328.

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NOTE

* Slijkhuis H and Marx AF. Microbiological preparation of 9α-hydroxy-17-keto steroids. Not yet published European Patent Application 87202619.0 by Gist-brocades.

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