New synthesis of estradiol from androsta-1,4-diene-3,17-dione

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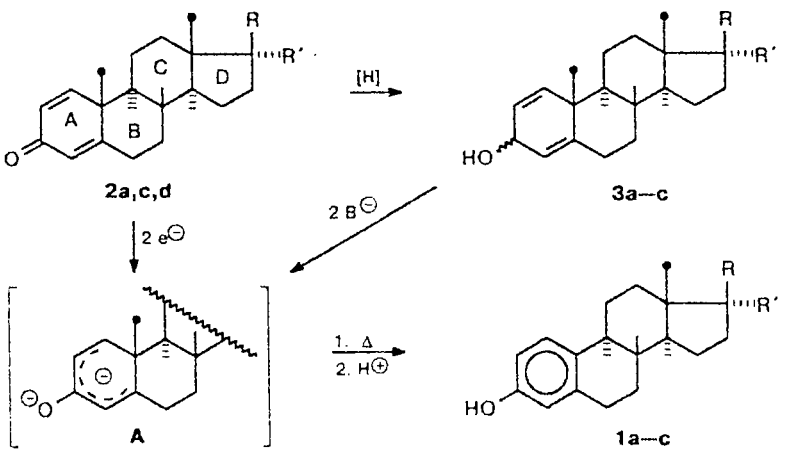
A new method for the aromatization of ring A in androsta-1,4-diene-3,17-dione, available from sterols by means of the microbiological degradation of the side chain, was developed. The method consists of the reduction of androsta-1,4-diene-3,17-dione to the corresponding dienediol followed by double C,O-deprotonation of ring A, accompanied by expulsion of the 19-methyl group and formation of estradiol in a high yield.

Key words: androsta-1,4-diene-3,17-dione, aromatization, angular demethylation, estradiol, partial synthesis.

Estradiol (**1b**) is the most important of female reproductive hormones (estrogens) and a valuable intermediate in the synthesis and production of other estrogens and a large number of steroid preparations with estrogenic (estradiol ethers, ethynylestradiol), gestagenic and anabolic (derivatives of 19-nortestosterone, prepared by reduction of the aromatic ring in estrogens), contraceptive (gestagen + estrogen), and antiimplantation (Mifepristone) activities.[1]

At present, estrogens are mainly obtained on the preparative scale by chemical synthesis from androsta-1,4-diene-3,17-dione (ADD, **2a**), available from microbiological transformation of β-sitosterol or cholesterol. ADD is converted into estrogens using two main methods; both of them produce primarily estrone (**1a**). One method is based on high-temperature pyrolysis of ADD under extreme conditions (500—700 °C, contact time up to 1 s).[2] In the other method, removal of the angular 19-methyl group from ADD (*i.e.*, the so-called aromatization of the steroid) is attained by the Dryden reaction.[3] The method consists of the treatment of ADD 17-ethyleneketal (**2c**) with sodium diphenylide in diglyme; this results in elimination of the 19-methyl group to give estrone 17-ethyleneketal (**1c**). The latter is converted into estrone, estradiol, and other estrogens by conventional procedures. Although both methods afford the products in relatively high overall yields (more than 60%), they have substantial limitations, due to the ne-

Scheme 1



R + R' = O (**a**); R = OH, R' = H (**b**); R + R' = OCH_2CH_2O (**c**); R = R' = H (**d**)

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 3, pp. 599—601, March, 1999.

1066-5285/99/4803-0593 $22.00

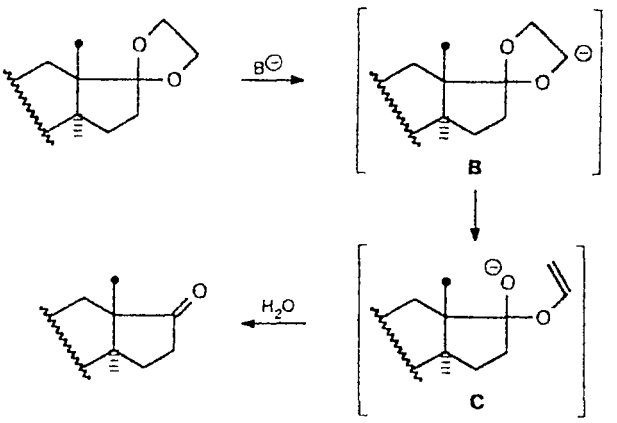
cessity of using special equipment, intermediate protection of the 17-oxygen function, *etc.*; therefore, alternative methods need to be developed.

Previously we showed[4] that the mechanism of the Dryden reaction is the addition of two electrons to the cyclohexadienone fragment of the ADD molecule (ring A) to give dianion **A**, which eliminates the 19-methyl group as a methyl anion to give the phenoxide of the corresponding A-aromatic steroid. This mechanism was confirmed by generating dianion **A** by an alternative route, namely, by double deprotonation (at oxygen and carbon) of steroid cyclohexadienol **3d**, which resulted in aromatization of ring A, similar to that occurring in the Dryden reaction. We employed this way of generation of type **A** dianions to transform ADD into estrogens.

To obtain the required cyclohexadienol group in ring A, the 3-carbonyl group in ADD is to be reduced. When lithium aluminum hydride was used as the reducing agent (this has been reported for ADD[5]), the 1,2- and 1,4-addition reactions occurred competitively; therefore the resulting (upon simultaneous reduction of the 17-carbonyl group) dienediol **3b** contained a considerable admixture of the corresponding Δ^4-3-ketosteroid, testosterone. The proportion of testosterone formed was 10—60% and increased when a larger excess of lithium aluminum hydride was used. Similar results were obtained when lithium diethoxyaluminum hydride[6] and aluminum hydride[7] generated *in situ* were used; in the case of sodium borohydride, only 1,4-addition products were obtained. A clean 1,2-addition was achieved by using sodium bis(2-methoxyethoxy)aluminum hydride[8] [$NaAlH_2(OCH_2CH_2OMe)_2$, Red-Al]. In this case, the yield of a mixture of 3-epimeric dienediols **3b** with 97% purity reached 98.6%, and the corresponding 17-ketosteroids were formed as the main impurity. The ratio of the epimers 3α-**3b** : 3β-**3b**, whose configurations were assigned by analogy with published data,[4] was 4 : 6. Separation of the 3-epimers is not required for their subsequent use. Similar results were obtained in the reduction of ADD 17-ethyleneketal (**2c**), resulting in a mixture of epimeric dienols **3c**.

The next, and more complicated, problem was to generate type **A** dianion from dienediol **3b**; this would require triple ionization of the molecule (double ionization of ring A and ionization at O(17)). The attempts to make this problem easier by using protective groups for the 3- and 17-oxygen functions (the 3-*O*-methyl ether, 17-ethyleneketal) resulted in unusual transformations of these "inert" groups under the severe alkaline conditions needed for this reaction. For example, treatment of ketal **3c** with a large excess (10 equiv.) of Bu^nLi in heptane in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) at 100—110 °C directly gave estrone **1a** as the major product (in up to 28% yield), resulting from aromatization of ring A with simultaneous cleavage of the ethyleneketal group. The probable mechanism of this cleavage is deprotonation of the $-OCH_2CH_2O-$ unit in the ethyleneketal group to give α,β-dialkoxy carbanion (**B**), which is converted upon rapid β-elimination into the O-anion (**C**); the latter species is the alkoxide derived from the 17-ketone vinyl hemiketal. Evidently, this alkoxide can be converted into the 17-ketone simply by treatment with water. However, the yield of the individual products of aromatization of dienediol **3b** derivatives decreases as a result of this and other side processes that accompany aromatization of ring A; therefore, unprotected diol **3b** should be considered the optimum intermediate compound.

Scheme 2



Study of the aromatization of dienediol **3b** showed that aromatization giving estradiol **1b** *via* the corresponding anion (trianion in this particular case) of type **A** occurs more or less efficiently with most of the bases studied (KH, PhNa, $NaPh_2$, Bu^nLi, $Bu^nLi \cdot TMEDA$, Pr^i_2NLi) and in various solvents (*n*-heptane, toluene, THF, diglyme, TMEDA, Et_2NH). It is the high-temperature stability of the base in the employed medium (which is low in ether and amine solvents) rather than the solubility of the intermediate type **A** trianion (or the preceding dialkoxide), which is always very poor, that proved to be the critical factor determining the high product yields in this reaction. Correspondingly, the aromatization proceeds most smoothly under the simplest conditions involving the reaction with Bu^nLi in toluene for 9—11 h at 100 °C. In this case, the yield of estradiol **1b** reached 84—86% when only 5 equiv. (167% of the amount required theoretically) of Bu^nLi was used.

The two-step procedure for the transformation of ADD into estradiol not only surpasses in experimental simplicity and efficiency (overall yield 83—85%) other analogous procedures for ADD transformation into estrogens but can also be used for aromatization of ring A in other steroids with a 19-methyl group, which previously could be performed only with significant difficulties.

Experimental

^{1}H NMR spectra were recorded on a Bruker WM-250 spectrometer (250.13 MHz). The optical rotations were measured at 100 °C on a DIP-360 instrument (JASCO, Japan). TLC analysis was performed on Kieselgel 60 (Merck) plates using the solvent systems indicated below; the spots were visualized by spraying the plates with a solution of $Ce(SO_4)_2$ in 10% H_2SO_4 with subsequent heating. Commercial ADD (Russia), m.p. 138.5—140°C, with a purity of >95%, 70% solutions of Red—Al in benzene (Chemapol, Czechoslovakia), and a 1.6 *M* solution of Bu^{n}Li in hexane (Fluka) were used.

Androsta-1,4-dien-3α/β,17β-diol (3b). A 70% benzene solution of Red—Al (8.8 mL, 26.5 mmol) was added to 25 mL of anhydrous toluene under argon. The resulting colorless transparent solution was heated to 50—55 °C, and a solution of ADD (5.0 g, 17.6 mmol) in 50 mL of anhydrous toluene was added in portions with stirring over a period of 15 min. The resulting milk-white thick paste was stirred for 1 h at 50—60 °C and cooled to ~20 °C, and 100 mL of a 20% aqueous solution of NaOH was added. The steroid precipitate was filtered off from the resulting suspension and washed on the filter with 250 mL of water (to pH 7 of the wash water). The crystals were dried *in vacuo* over KOH (2 h at 50—60 °C and 12 h at 20 °C (2 Torr)) to give 4.45 g (87.8%) of diol **3b** as a while crystalline powder, purity >99%, ratio of epimers 3α : 3β = 4 : 6 (NMR data), m.p. 198—200 °C, $[\alpha]_D^{23}$ +33.8° (*c* 0.80, dioxane), R_f 0.18 (β-epimer) and 0.32 (α-epimer) (EtOAc—hexane, 1 : 2 + 0.1% triethylamine, four runs) (see Ref. 5). ^{1}H NMR ($CDCl_3$), δ: 0.79 (s, 3 H, C(18)H_3); 1.07 (s, 1.8 H, C(19)H_3 in the 3β-epimer); 1.12 (s, 1.2 H, C(19)H_3 in the 3α-epimer); 3.64 (t, 1 H, H(17), J = 8.3 Hz); 4.50 (br.s, 1 H, H(3)); 5.52 (m, 0.6 H, H(4) in the 3β-epimer); 5.55 (m, 0.4 H, H(4) in the 3α-epimer); 5.79 (br.d, 1 H, H(2), J = 10.7 Hz); 5.97 (d, 0.4 H, H(1) in the 3α-epimer, J = 10.7 Hz); 6.00 (d, 0.6 H, H(1) in the 3β-epimer, J = 10.7 Hz). The upper (toluene) layer of the two-layer filtrate was separated, the aqueous layer was extracted with toluene (3×15 mL), and the toluene solutions were combined, dried with Na_2SO_4, and evaporated to dryness. The resulting white crystalline precipitate (0.55 g, 10.8%) contained 70—75% diol **3b**; a mixture of epimeric androsta-1,4-dien-3α/β-ol-17-ones (**3a**) was formed as the main impurity (up to 25%). This impurity was responsible for additional signals in the ^{1}H NMR sprectrum of the product ($CDCl_3$), δ: 0.91 (s, 3 H, C(18)H_3); 1.10 (s, 1.8 H, C(19)H_3 in the 3β-epimer); 1.15 (s, 1.2 H, C(19)H_3 in the 3α-epimer). R_f 0.13 and 0.27 (the same system). Combining the two portions of the product gives 5.00 g (98.6%) of diol **3b** of 97% purity (the above-mentioned impurity accounted for 3%), which was used in the next step without additional purification.

Estra-1,3,5(10)-triene-3,17β-diol (estradiol, 1b). A 1.6 *M* solution of Bu^{n}Li (16.3 mL, 26 mmol) in hexane was added under argon at 60 °C over a period of 20 min to a stirred suspension of diol **3b** (1.5 g, 5.2 mmol) in 25 mL of anhydrous toluene. A bright-orange suspension formed. The temperature of the bath was raised to 100 °C with constant removal of hexane vapor from the flask, and the suspension was stirred at this temperature for 11 h; the disappearance of the starting compound was monitored by TLC of samples taken from the reaction mixture and acidified (EtOAc—hexane, 3 : 2, double development, R_f 0.23 and 0.39 (yellow spots, the epimers of diol **3b**), 0.52 (red spot, estradiol **1b**), 0.67 (yellow spot, the products of acid catalyzed 3-monodehydration of diol **3b**)). The brown-yellow suspension was cooled to ~20 °C, and 14 mL of water and 8 mL of 18% HCl (to pH 1—2 of the aqueous layer) were successively added to it with stirring. The precipitate of the steroid formed at the toluene—water interface was filtered off, washed with 5 mL of water, and dried *in vacuo* over KOH to give 1.08 g (76.2%) of estradiol **1b** as a beige powder. An additional portion of the same product (0.11 g, 7.6%) was isolated by alkaline extraction of the toluene layer. The total yield of estradiol **1b** was 1.19 g (83.8%), purity ≥95% (NMR data). Recrystallisation from 50% aqueous ethanol with clarification by activated carbon gave estradiol **1b** in 90% yield with >99% purity as a white coarsely crystalline powder, m.p. 175—176 °C (*cf.* Ref. 2). ^{1}H NMR (DMSO-d_6), δ: 0.67 (s, 3 H, C(18)H_3); 1.0—2.3 (m, 13 H, H(8+9) + C(7+11+12+14+15+16)H_2); 2.68 (m, 2 H, C(6)H_2); 3.54 (t, 1 H, H(17), J = 8.6 Hz); 6.44 (s, 1 H, H(4)); 6.50 (dd, 1 H, H(2), J = 2.2 and 9.4 Hz); 7.04 (d, 1 H, H(1), J = 9.4 Hz).

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Received October 29, 1998