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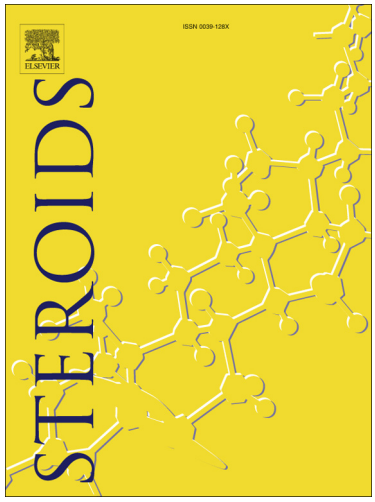

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Thermodynamic Meerwein-Ponndorf-Verley reduction in the diastereoselective synthesis of 17α-estradiol

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Abstract

The synthesis of 17α-hydroxy steroids generally requires multistep synthetic manipulations. 17α-estradiol is no exception as it also involves protection and release of the 3-hydroxy functional group. The diastereoselective reduction of the 17-keto-steroid can be utilized to prepare 17α-hydroxy-steroids. 17α-estradiol was synthesized under thermodynamic Meerwein-Ponndorf-Verley (MPV) conditions in a single step starting from commercially available estrone, followed by a simple chromatographic separation over silica gel. The remaining mixture of unreacted estrone and estradiols can be easily recycled by Oppenauer oxidation to estrone, leading to an overall yield of 68% of 17α-estradiol.

Keywords: Estradiol-α, Meerwein-Ponndorf-Verley reduction, steroid

1. Introduction:

Estrogens are prescribed for hormone replacement therapy to manage menopausal symptoms such as hot flashes and vaginal dryness and they can also be taken for the prevention of osteoporosis [1-4]. A commonly used estrogen drug, Premarin™, contains about ten different biologically active estrogens [5], including estrone (**2**), 17β-estradiol (**3**) and 17α-estradiol (**1**). 17α-estradiol exhibits potential activity in menopause management and is also implicated in having a beneficial influence on the central nervous system [6]. The synthesis of 17α-hydroxy steroids is challenging and most of the synthetic efforts have been devoted to the inversion of the 17β-hydroxy steroids by means of the Mitsunobu reaction [7, 8] and displacement of sulfonyl ester [9-12]. Both of these methods involved multiple steps, protection and removal of the protecting group and expensive or custom-synthesized reagents. We have been looking for a cheap, quick and economical way to manufacture large quantities of 17α-estradiol. Herein, we are reporting one-step synthesis of 17α-estradiol starting with readily available estrone (**2**) by means

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of the reduction of the 17-carbonyl steroid with aluminum alkoxides (MPV reduction) [13-15] at an elevated temperature instead of the inversion of the hydroxyl function. Aluminum isopropoxide has been reported for the reduction of estrone methyl ether [16] under kinetic conditions with modest yield without the mention of any stereochemical outcome.

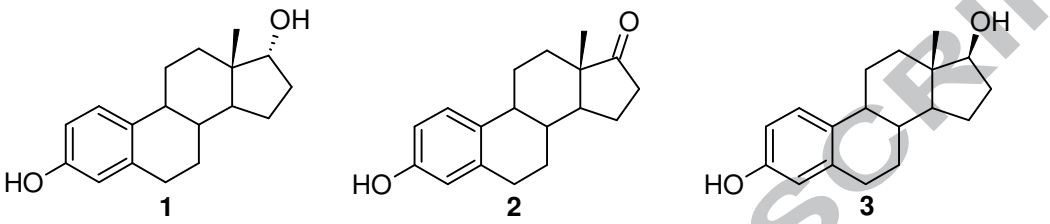

Figure 1. Estrogens: 17α-estradiol (**1**), estrone (**2**) and 17β-estradiol (**3**).

2.0 Experimental

2.1 General methods

Nuclear magnetic resonance spectra were recorded on a Bruker ARX (300 MHz) spectrometer as deuterochloroform ($CDCl_3$) solutions using tetramethylsilane (TMS) as an internal standard (δ = 0) unless noted otherwise. High-performance liquid chromatography was performed on Waters Alliance 2695 with photodiode array 2996 systems on a Waters XTerra® RP_{18} column (3.5 µm, 4.6 x 150 mm). "Flash column" chromatography was performed on 32-64 µM silica gel obtained from EM Science, (Gibbstown, NJ). Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 cm x 10 cm with 250 µM layer and pre-scored). Most chemicals and solvents were analytical grade and used without further purification. 17α-estradiol was purchased from Aldrich Chemical Company (Milwaukee, WI), 17β-estradiol was purchased from Berlichem Inc. (Wayne, NJ), estrone was purchased from Spectrum Chemical MFG Corp. (New Jersey) and aluminum isopropoxide and aluminum s-butoxide were purchased from Strem Chemical Inc. (Newburyport, MA). All known products were compared with their authentic samples and their reported spectroscopic data.

2.2 Synthesis

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*17α-estradiol (**1**)*

In an oven-dried 5-L four-necked RBF fitted with mechanical stirrer and a condenser under nitrogen, estrone (100 g) was added followed by the additions of 2-methyltetrahydrofuran (1.0 L) and 2-pentanol (80 mL, 2.0 eq.) and this clear mixture was heated to reflux. The mixture of estrone at reflux became a clear solution as aluminum s-butoxide (225 g, 2.47 eq.) was added with the help of a Teflon tube cannula (5 mm wide) over 20 minutes at reflux. The reaction was refluxed for 18 hours with stirring, cooled to room temperature, cooled in an ice bath before being quenched with 20% aq. sodium hydrogen sulfate hydrate (400 g in 2.0 L water, pH 2-3), diluted with ethyl acetate (1.0 L) and stirred for 30 minutes to dissolve all the solids. The pH of the aqueous layer was 4. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (1 L). The combined organic layers was dried (sodium sulfate), concentrated, and the crude was further dried on a vacuum pump overnight. The crude HPLC showed a 45:55 α/β estradiol ratio with 4% estrone starting material. The mass of the crude was about 110 g (110%).

The crude was dissolved in THF (1 L), adsorbed on silica gel (400 g, 4 g silica gel per gram of estrone) and dried under vacuum. The large column containing 2.5 kg of silica gel was equilibrated with DCM (12 L), the adsorbed crude was loaded with some sand on top and it was run with the following solvents: (1) 6 L DCM, (2) 4L 3% THF in DCM, (3) 4L 4% THF in DCM, (4) 4L 5% THF in DCM, (5) 12L 6% THF in DCM. The fractions were collected in 500-mL portions. The concentration of the fractions yielded 17α-estradiol as a white solid **1** (23.5 g, 99+%, mp 221.0 – 221.5°C (lit. 219-222°C [17]), $[\alpha]_D^{21}$ +52.8 (c= 1.06, dioxane) (lit. +52.9U±1 (c=0.9%, dioxane) [18])), estrone **2** (2.0 g) and a mixture of 17α/β-estradiol (74 g, 1:4 α:β).

*Estrone (**2**)*

The estradiols (mixture of α/β; 74 g, 1 eq.) were first dissolved in 2-methyltetrahydrofuran (390 mL) at room temperature under nitrogen with stirring. Benzaldehyde (83.6 g, 2.9 eq.) and 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT, 1.5 g, 0.025 eq.) were added and dissolved. Aluminum isopropoxide (27.7 g, 0.5 eq.) was then added and the resulting light brown solution/slurry was stirred for 4 hours at ambient temperature. The crude HPLC showed 95% conversion. The reaction was quenched with aq. sodium hydrogen sulfate (70 g in 1 L water), extracted with ethyl acetate (2 x 400 ml) and the combined organic layer was dried over sodium sulfate, filtered and concentrated. The HPLC showed 95% of the desired product **1**. The residue was dissolved in DCM (100 mL) and triturated with hexanes (700 mL); the

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white product was collected on a filter paper and washed with hexanes (3 x 40 mL) to yield 43.3 g of estrone **2** (58 %). There was more product in the mother liquor which needed to crystallize or be chromatographed. The presence of excess benzaldehyde and benzyl alcohol formed during the reaction causes the lower recrystallization of the product.

3. Results and discussion

Traditional reducing agents such as sodium borohydride and lithium aluminum hydride were initially employed at ambient temperature to monitor the scope of the diastereospecificity in the direct reduction of estrone to estradiol (Table 1). It is evident from the literature as well as entry 1 and 2 that under the kinetic reaction conditions, 17β-estradiol is produced predominantly due to the addition of hydride from the less steric alpha face of estrone. A modest yield of 17α-estradiol was encouraging (entry 3) when sodium borohydride was added to the reaction at refluxing estrone in isopropanol. In light of this outcome, we envisioned that a suitable reducing agent under thermodynamic conditions will result in the formation of a 17α-estradiol/17β-estradiol mixture directly from estrone with an excellent yield. With modern separation techniques such as process HPLC becoming more and more popular, one-step synthesis followed by chromatographic separation could be an attractive alternative to the tedious and low-yield stereoselective synthesis.

In order to test our hypothesis, we turned our attention to the Meerwein-Ponndorf-Verler (MPV) reduction which generally employs aluminum isopropoxide for chemoselective reduction of aldehydes and ketones to alcohols [13-15]. The addition of aluminum isopropoxide (0.5 eq.) to refluxing estrone in isopropanol resulted in a 3:7 α/β ratio of 17-hydroxy-estradiol (entry 4) with only 7% conversion. The increase in the α/β ratio of the hydride addition was a step in the right direction, even though the conversion was low, which was addressed by adjusting the use of stoichiometric quantities of the reducing agent (entry 5, 6, 7). The best yield (83%) was achieved with toluene as a solvent which boils at a higher temperature (110^{o}C) than isopropanol (82^{o}C) with a product ratio of 1/3 in favor of 17α-estradiol (entry 7).

It is evident that running this reaction under thermodynamic MPV conditions by increasing the reaction temperature is beneficial on the reaction yields as well as the desired product ratio. Acetone was produced during the reaction with aluminum isopropoxide as the products were forming, which caused the internal reaction temperature to be lower than the solvent boiling temperature resulting in lower yields and product ratio. Aluminum s-butoxide was next tried as a reducing agent (entry 8) which

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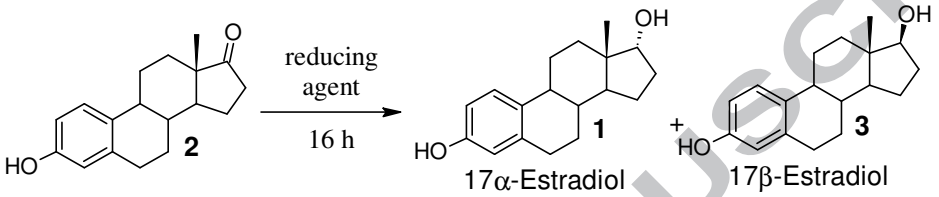
resulted in an 83% conversion with 44% desired product. It is worth noting that in this reaction, butanone is produced which has a higher boiling point than acetone. The use of excess reducing agent improved the conversion but did not affect the product ratio (entry 10) and the higher boiling solvent such as xylenes also pushed the product yields to 99% but did not influence the product ratio (entry 11). It is also worth mentioning that the regular silica gel chromatography is sufficient to separate the two diastereoisomers of the estradiol.

Table 1:
Exploring reaction conditions for diastereoselective reduction of estrone **2**



Entry	Reductant (equivalent)	Temperature	Additive	Solvent	17α-estradiol	17β-estradiol	Conversion (Estrone)
1	$NaBH_4$ (1.5)	rt	^{i}PrOH (5 mL)	MeOH (5 mL)	<1	~99	100% (0)
2	$LiAlH_4$ (5.0)	rt	-	THF (20 mL)	1.8	98.2	100% (0)
3	$NaBH_4$ (2.0)	reflux	-	^{i}PrOH (5 mL)	4.6	95.4	100% (0)
4	$Al(O^{i}Pr)_3$ (0.5)	reflux	-	^{i}PrOH (10 mL)	29	71	7% (93)
5	$Al(O^{i}Pr)_3$ (2.2)	reflux	-	^{i}PrOH (10 mL)	29	71	55% (45)
6	$Al(O^{i}Pr)_3$ (2.5)	reflux	^{i}PrOH (1 mL)	Toluene (5 mL)	29	71	80% (20)
7	$Al(O^{i}Pr)_3$ (2.8)	reflux	-	Toluene (5 mL)	32	68	83% (17)
8	$Al(O^{i}Bu)_3$ (1.3)	reflux	-	Toluene (10 mL)	44	56	83% (17)
9	$Al(O^{i}Bu)_3$ (1.8)	reflux	-	Toluene (10 mL)	43	57	83% (17)
10	$Al(O^{i}Bu)_3$ (2.5)	reflux	-	Toluene (10 mL)	44	56	97% (2)
11	$Al(O^{i}Bu)_3$ (2.5)	reflux	-	Xylenes (10 mL)	44	56	99% (1)

Once the conditions were optimized, the large-scale synthesis of 17α-estradiol was commenced starting with 100 g of estrone according to the conditions as in entry 10. It was to our surprise that the reaction became a solid precipitate a few minutes after the addition of aluminum s-butoxide with solvent which separated out and a mechanical stirrer which stopped working. It was assumed that the unprotected 3-hydoxyl and 17-hydroxyl of the newly produced estradiols formed oxygen-aluminum-oxygen bonds (insoluble solid polymer) which had caused the mechanical stirrer to fail. Even with no stirring, the reaction products' outcome was similar as on a small scale. Further optimization led us to use 2-methyltetrahydrofuran, a more polar solvent than toluene and a higher-boiling additive co-solvent

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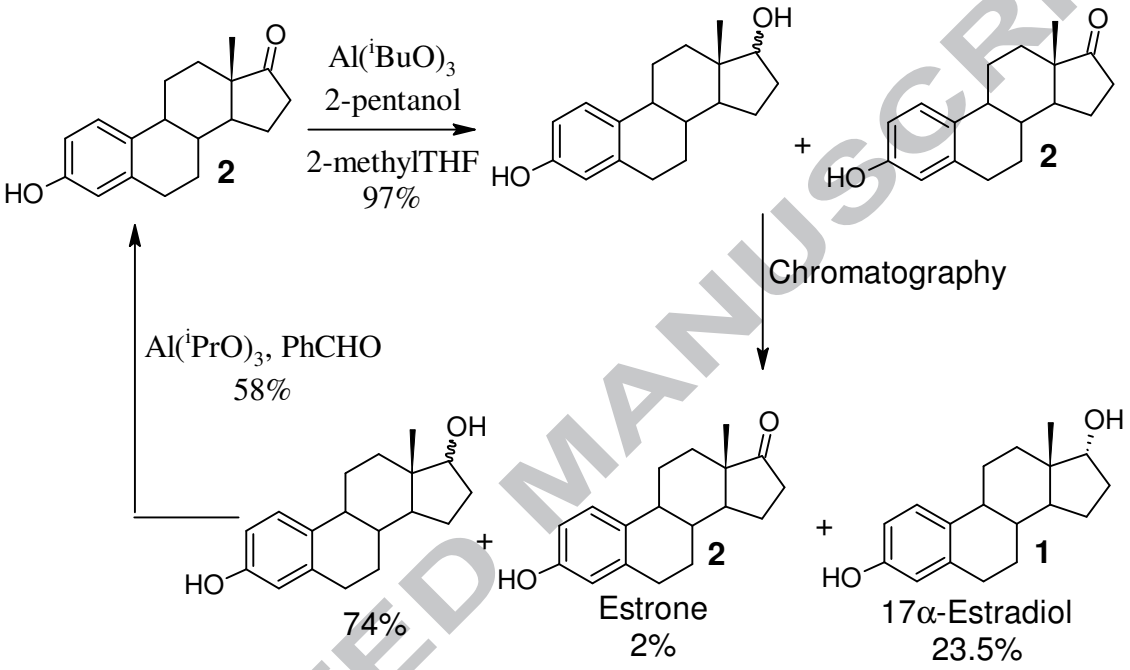
2-pentanol which was capable of interrupting the formation of the polymer. Under these conditions, the large-scale reaction with 100 g of estrone worked without stirring failure. The crude HPLC showed 45:55 α/β estradiol ratio with 4% estrone starting material. The purification of the crude residue under regular silica gel chromatography yielded 23.5% with estrone (2%) and a mixture of estradiols (74%). We believe that improvement in chromatographic techniques would lead to even higher amounts of 17α-estradiol from this reaction.



Scheme 1: Estrone-estradiol reduction and oxidation

The mixture of estradiols, predominantly 17β-estradiol, can be recycled back to estrone under Oppenauer oxidation conditions with benzaldehyde as the sacrificial oxidant instead of acetone. Thus, estradiols were treated with aluminum isopropoxide in the presence of benzaldehyde in 2-methyltetrahydrofuran at ambient temperature to yield estrone at a 58% yield after direct recrystallization from the crude reaction mixture. The total yield for a 17α-estradiol base on recycled estrone was 68%.

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4. Conclusion

In conclusion, we have demonstrated a convenient and inexpensive way to synthesize 17α-estradiol in a single chemical step without the use of any protecting group on the starting estrone by utilizing thermodynamic MPV conditions with commercially inexpensive reagents on a large scale. The higher boiling 2-butanone produced during reaction under atmospheric pressure from aluminum s-butoxide compared to acetone produced from aluminum isopropoxide resulted in a higher reaction temperature and led to a higher product ratio as well as product yields. A simple silica gel chromatographic technique is employed for the separation of the diastereoisomers. The undesired estradiol mixture can be easily recycled back to estrone with inexpensive reagents for reuse, resulting in less waste.

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Highlights:

The synthesis of 17α-Hydroxy-steroids generally requires multistep synthetic manipulations. 17α-Estradiol is no exception which also involves protection and release of 3-hydroxy functional group. The diastereoselective reduction of the 17-keto-steroid can be utilized to prepare 17α-hydroxy-steroids. 17α-Estradiol was synthesized under thermodynamic Meerwein-Ponndorf-Verley (MPV) conditions in a single step starting from commercially available estrone, followed by a simple chromatographic separation over silica gel. The remaining mixture of unreacted estrone and estradiols can be easily recycled by Oppenauer oxidation to estrone, leading to an overall yield of 68 % of 17α-estradiol.

- 17α-Estradiol was synthesized under thermodynamic Meerwein-Ponndorf-Verley (MPV) conditions in a single step starting from commercially available estrone.
- 17α-Estradiol was isolated from the mixture of 17α/β-estradiol mixture by a simple chromatographic separation over silica gel.
- The remaining mixture of unreacted estrone and 17α/β-estradiol can be easily recycled by Oppenauer oxidation to estrone, leading to an overall yield of 68 % of 17α-estradiol.

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