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Advanced Synthetic Strategies for Progesterone: Combining *trans*-Hydrogenation and Rupe's Rearrangement of 4-Androstenedione for Enhanced Yield

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Progesterone, a steroid hormone, plays an important role in the human body, especially in the reproductive system. A unique, minimal, cost-effective and high yielding organic synthesis protocol for the synthesis of progesterone has been established. The proposed economical route utilizes low-cost and easily available hormone intermediate 4-androstenedione and acetylene gas, offering unique pathway to synthesize progesterone, which can be further scaled to the commercial level. The process starts with the synthesis of a propargylic alcohol intermediate from 4-androstenedione, yielding 93% through an improved procedure. Subsequently, this intermediate undergoes Rupe's rearrangement, catalyzed by copper over an alumina catalyst, to form an enyne intermediate, which is then rehydrated to produce an enone with a 77% yield. The final step involves the selective reduction of an olefin bond *via* palladium-catalyzed *trans*-hydrogenation reaction, resulting in progesterone with a 75% yield. The final product is of pharmaceutical-grade purity as confirmed by HPLC analysis.

Keywords: 4-Androstenedione, Rupe's rearrangement, Progesterone, Palladium, *trans*-Hydrogenation.

INTRODUCTION

In human body, hormones are highly significant naturally occurring biochemical substances that act as genetic transmitters between cells and organs and are essential for controlling a number of body functions. Importantly, progesterone and estrogen are the female sex hormones, which generate in the body majorly after ovulation by the corpus luteum and a small amount is biosynthesized in adrenal glands and in the placenta during pregnancy [1]. Progesterone is physiologically important due to its actions as genomic and non-genomic receptor mechanisms and on tissues such as mammary gland, endometrium, cardiovascular system, peripheral and central nervous systems and bones alongside the preservation of pregnancy [2,3]. Earlier works on the synthesis of progesterone from diosgenin, which was extracted from *Dioscorea tokoro* and later from *Dioscorea mexicana* plants are also reported in the literature [4]. Estrogen plays significant role in human body from puberty to menstruation and pregnancy to menopause, while progesterone supports pregnancy by aptly preparing the uterus lining to keep fertilized egg intact [5].

Biosynthesis of progesterone follows transformation of cholesterol to pregnenolone through the side-chain cleaving cytochrome P450scc, which is present in the inner membrane of the mitochondria [6]. The translocase ligands that are responsible for bringing the cholesterol from outer to the inner mitochondrial membrane are also increasing the biosynthesis of pregnenolone, which later converted into progesterone by the action of hydroxysteroid dehydrogenase [7]. Apart from many other biosynthetic ways, progesterone is alternatively chemically prepared through several ways. Ever since its isolation in 1934, Riegel & Prout in 1948 reported the synthesis of radioprogesterone, progesterone-21-C_{14}, an acetyl carbon labelled progesterone from the corresponding chloroformyl derivative 3-keto-4-etiocholenyl chloride with carbon labelled dimethylcadmium [8]. Ott *et al*. [9] reported a practical synthesis of progesterone in much improved overall yield of 37% starting from ergosterol following a multistep approach involving stigmastadienone. Schumacher *et al*. [10] reported the synthesis of progesterone from cholesterol by the actions of cytochrome P450scc and hydroxysteroid dehydrogenases in a sequential manner. Contrary to the biological importance of progesterone,

water-soluble progesterone-conjugated probes were developed to efficiently image the hormone related cancer diagnosis using magnetic resonance method [11]. Similarly, several fluorescent progesterone receptor antagonists possessing varied functional groups and solubility have been developed [12]. In view of various applications of progesterone, an easy and cost-effective methods for its chemical synthesis are of great importance. In this work, the chemical synthesis of progesterone is reported in high yields from 4-androstenedione, which is also a naturally occurring androstane steroid.

EXPERIMENTAL

All solvents used in this study such as dimethyl sulfoxide, 1,4-dioxane, toluene, ethanol, dichloromethane, ethyl acetate, *n*-hexane, *etc.*, were procured from commercial sources and were used with appropriate drying procedures using standard drying agents under an extra pure nitrogen atmosphere. All solvents required for HPLC analyses were of HPLC grade and used without further purification. All chemicals required for this method development such as sodium methoxide, 4-androstenedione, copper sulphate pentahydrate, neutral alumina, methanesulfonic acid, trifluoromethanesulfonic acid, palladium over carbon, triethyl orthoformate, formic acid, *etc.*, are purchased from commercial sources such as Merck Pvt. Ltd., SD Fine Chemicals, *etc.*, Agilent liquid chromatograph equipped with 1200 autosampler with UV-Vis detector and appropriate integration software HPLC was used for the analyses.

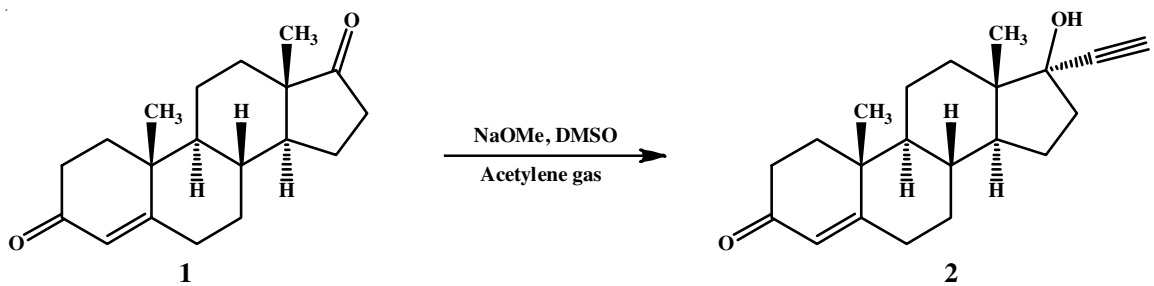
Synthesis of propargylic alcohol intermediate: In a 500 mL four-necked round bottom flask, 30.8 g of sodium methoxide and 110 mL of DMSO were mixed. The air was displaced by nitrogen bubbling. Acetylene was bubbled into the mixture for 6-8 h through gas sparger with stirring at temperature of 15 to 25 ºC. The bubbling was conducted carefully to prevent the release of acetylene into the atmosphere and then 80 mL toluene solution of 4-androstenedione (40 g) was added slowly over 80 min maintaining at the same temperature. Acetylene bubbling to the flask was continued until completion of the reaction, which was established by TLC. Later, the acetylene flow was stopped and then nitrogen bubbled through the tube. A 100 mL of water was added at 25 ºC for 30 min with constant stirring during which the temperature rose to 35-40 ºC. The pH of the reaction mixture was adjusted to 3.5-5.5 using dilute H_2SO_4 acid (18 mL concentrated in 400 mL water) to obtain a yellowish suspension which was stirred for 1 h at 20-25 ºC. Further, 100 mL of water was added over 2 h with stirring and the mixture aged by stirring for further 1 h at 25 ºC. Finally, formed precipitate was filtered and washed with water until the pH of the filtrate became 6-7, dried under vacuum at 70 ºC (**Scheme-I**).

The, obtained crude product **2** was purified by recrystallization method. In a 500 mL flask, 42.0 g of propargylic alcohol and 80 mL of 1,4-dioxane was mixed. Air was displaced by applying at least 3 nitrogen/vacuum cycles and the reaction mass was heated to 100 ºC to ensure complete dissolution. If turbidity observed, the temperature was adjusted between 80 and 90 ºC and the solution was filtered. The filtrate was washed with 20 mL of 1,4-dioxane and the solution was cooled to room temperature. Then stirred the solution for at least 1 h and the suspension was filtered and washed with cold 1,4-dioxane, dried under vacuum below 70 ºC.

Synthesis of pregna-4,16-diene-3,20-dione intermediate: The copper alumina catalyst was prepared by stirring 40 g of $CuSO4{\cdot}5H_2O$ and 160 mL of water at room temperature until a clear solution was obtained. Then 20 g of neutral alumina was charged into the flask and the mixture was further stirred for 60 min. 1,4-Dioxane (150 mL) was added to give a pale blue slurry with a virtually colourless supernatant, which was then discarded. The solution was dried under suction and the solid was transferred to a vacuum oven and dried at 60 ºC.

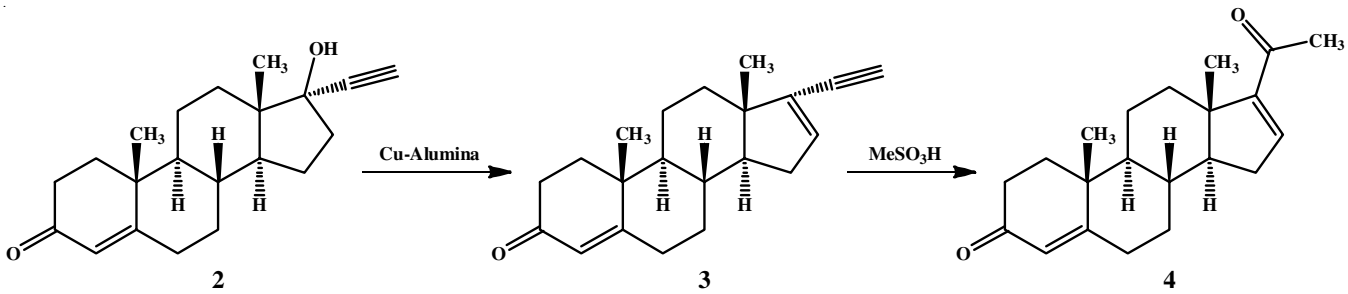
In 500 mL four-necked glass vessel, 250 mL of 1,4-dioxane was charged. Propargylic alcohol (**2**, 40 g) and copper sulfate on alumina (10 g) were added and the charging ports and lines were washed with an additional 25 mL of 1,4-dioxane. The system was purged with nitrogen for 30 min and the temperature was gradually increased from 25 ºC to 80 ºC. Once the reaction mass reached 80 ºC, methanesulfonic acid (2 mL) was added and the mixture was heated to reflux (~105 °C). The reaction was maintained at this temperature for 4-5 h and the reaction mass was cooled to 60 ºC and then filtered through a pad of celite, washing with 1,4-dioxane (20 mL). The mixture was concentrated at 50 ºC under vacuum in rotary evaporator to remove approximately 100 mL volume. Then, 200 mL of water was added at 50 ºC, resulting in the immediate precipitation of product. The resulting slurry was stirred at 40 ºC for 30 min and then left for 3-5 h. The solid was isolated by filtration washed with water and dried under suction. The resulting product **3** was used directly in the next stage (**Scheme-II**).

The enyne derivative **3** and 30 mL of acetic acid were charged in a clean and dried 2 L glass vessel with continuous nitrogen purging for 30 min at room temperature. Separately, a solution of trifluoromethanesulfonic acid (1.0 mL) in distilled water (5 mL) was added to the reaction mixture and then heated



1 **2**

Scheme-I



2 3 4

Scheme-II

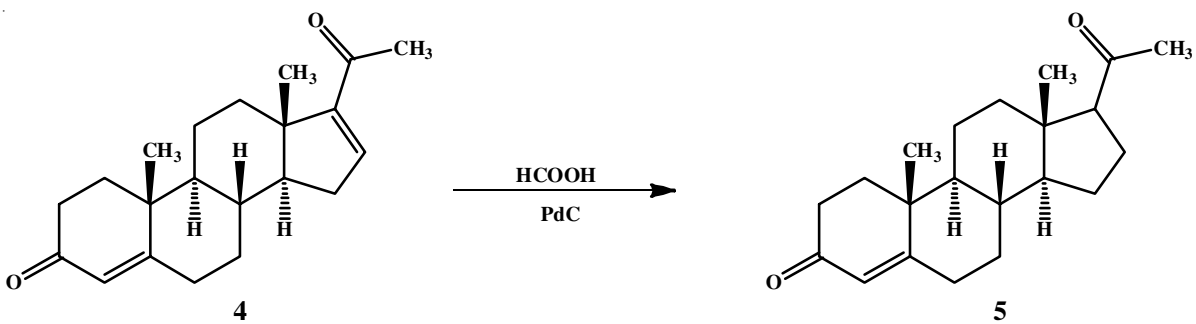
to 110 ºC. The reaction was maintained at this temperature until TLC analysis showed the complete consumption of enyne to get enone derivative **4** (**Scheme-II**). On completion of the reaction the solution was cooled to room temperature.

Synthesis of progesterone from enone by palladium catalyzed *trans*-hydrogenation reaction: A mixture of enone derivative **4** (10 g), Pd/C catalyst (100 mg) and toluene (50 mL) was stirred at room temperature under nitrogen atmosphere for 10 min. Triethyl orthoformate (15 mL) was added and the mixture was heated at 60 ºC followed by the addition of triethylamine (5 mL) and formic acid (2.0 mL). The reaction mixture was heated at 70-75 ºC for 15-16 h and then cooled to room temperature. The reaction mass was then filtered through a bed of celite (washing with toluene) to afford clear filtrate, washed with 1 M HCl and water. The reaction mass was washed with 20 mL of methanol followed by 15 mL of water and then filtered (**Scheme-III**). The recrystallization was done in 60% methanol to get a pure progesterone above 99.2% purity. The final synthesized compound was analyzed against USPNF_ M69870_02_01.

RESULTS AND DISCUSSION

Progesterone can be chemically synthesized in several ways following multi-steps reactions however, the simple and cost-effectiveness separation procedures to obtain the target in pure form are the vital prerequisites. Especially, isolation of progesterone as an active pharmaceutical ingredient (API) is significant as any trace impurities or byproducts may cause serious issues in pregnant women. Therefore, synthetically concise process to access API grade progesterone in high yield at large scale is of most essential research area in targeted organic synthesis active pharmaceuticals.

Starting from 4-androstenedione (androst-4-ene-3,17-dione), progesterone is synthesized with good yield using three simple step reactions (**Schemes I-III**). A DMSO solution of sodium methoxide was bubbled with acetylene gas over a period of 7-8 h under an inert atmosphere maintaining a temperature between 10-20 ºC. This reaction is expected to form sodium acetylide (NaC≡CH) along with the liberation a hydrogen molecule, which escapes through the acetylene vent. Further, a toluene solution of 4-androstenedione (**1**) was slowly added to sodium acetylide solution over a period of around 1-2 h maintaining the same temperature as the addition is exothermic in nature. However, during the complete addition and further reaction, the acetylene flow kept on and the completion of reaction was monitored by TLC until the concentration of 4-androstenedione (**1**) was < 1%, which approximately completes in 10 h. After this stipulated time, acetylene flow was stopped and instead nitrogen was bubbled for another 30 min and water was added dropwise over a period of 30 min at room temperature, while the temperature increases up to 40 ºC. Furthermore, using dilute H_2SO_4, the pH of the reaction mixture was adjusted to 4.0 during which the caution to be taken about the dissolved unreacted acetylene gas that emits out due to an exothermic reaction. Later, the reaction mixture was stirred at 25 ºC for additional 1-2 h and checked on TLC for the absence of an intermediate 17α-ethynil-17-hydroxy-andros-5-ene-3-one and water was added and stirred to age over a period of 2 h. Finally, the product precipitated was washed with water until the pH reaches 6.5 to 7 and was filtered under vacuum and dried at 70 ºC to obtain propargylic alcohol intermediate **2**, with a purity of 96.50%. Additionally, thus obtained product **2** was subjected to recrystallization using 1,4-dioxane in inert atmosphere and precipitated at 10-15 ºC to the yield of 93% with a much increased purity of up to 98.0%.



4 5

Scheme-III

Propargylic alcohol intermediate **2** is converted into corresponding pregna-4,16-diene-3,20-dione intermediate (enone) following an enyne intermediate formation. During this step, propargylic alcohol was transformed into an enone through a chemical rearrangement of functional groups at the C17 position known as copper catalyzed Rupe's rearrangement consists of dehydration and rehydration steps. In the dehydration step, an enyne **3** was yielded, while the subsequent rehydration of the enyne affords the enone product **4**. In Rupe's rearrangement step, copper catalyst was prepared by treating copper(II) sulphate pentahydrate in water with alumina at 20 ºC for 1 h. Upon 1,4-dioxane addition to the mixture and stirred for 18-20 h at the same reaction condition to obtain the product was dried in a vacuum oven at 60-65 ºC.

Both, propargylic alcohol and alumina supported copper catalyst in 1,4-dioxane were charged in a reaction vessel and the reaction mixture was purged with nitrogen for 30 min while slowly increasing to 80 ºC maintained under inert atmosphere. Further, methanesulfonic acid was added and the mixture was set to reflux at roughly 100-105 ºC and the reaction was continued until complete (~4 to 5 h). Thereafter, the reaction was cooled to 60 ºC and then filtered through a bed of celite, washed with fresh 1,4-dioxane and the solution was reduced to half of its volume under reduced pressure. In a concentrated solution, water was added up to 20 times to result in a precipitate that was stirred at 40 ºC for 30 min and cooled to room temperature to afford enyne intermediate **3**.

Consequently, the hydration of enyne product **3** using acidic condition afforded enone **4**, which is a known transformation majorly catalyzed by aluminium cation-radical recently reported by Moses *et al*. [13] or by following classical Brønsted acid and Kucherov-type alkyne hydration protocols [14]. Using protic acids which easily overcome the activation barrier constraint, this atom economic organic reaction has been documented [15,16]. Following similar procedure, enyne derivative **3** in acetic acid was stirred for 30 min at 21 ºC under inert atmosphere. To this mixture was added trifluoromethane sulfonic acid in water and stirred at 110 ºC for 3 h until complete consumption of enyne. The obtained slurry was stirred at 30 min at <15 ºC with a overall yield of 77%.

In the final step, progesterone **5** was synthesized from the respective enone derivative **4** using a palladium-catalyzed trans-hydrogenation process. A solution of enone derivative **4** in toluene was prepared. To this solution, 4 wt.% of palladium catalyst was added. The palladium catalyst was supported on carbon to assist the *trans*-hydrogenation reaction. The reaction was carried out under an inert atmosphere and the reaction mixture was gradually increased to 60 ºC. At this point, triethyl orthoformate was added to the reaction mxiture followed by the addition of formic acid and then stirred continuously at 60-70 ºC for 8 h. During this period, enone derivative **4** underwent complete conversion to progesterone (**5**). Monitoring the reaction showed that the conversion was complete within the specified time with the final yield of 75%.

Characterization: The synthesized progesterone from the above process was characterized against the reference standard procured from United State Pharmacopeia (Cat. no. 1568007) (Figs. 1 and 2). The structural analysis conducted through ^{1}H NMR and ^{13}C NMR also demonstrated the identical structures between the synthesized progesterone and the reference standard. Furthermore, mass spectrometry confirmed that the molecular mass of the synthesized progesterone matched that of the reference standard. However, multiple impurities and related substances, including impurity-B, impurity-C and impurity-G were anticipated to be present in the progesterone sample. The content of impurity B ranged from 0.11% to

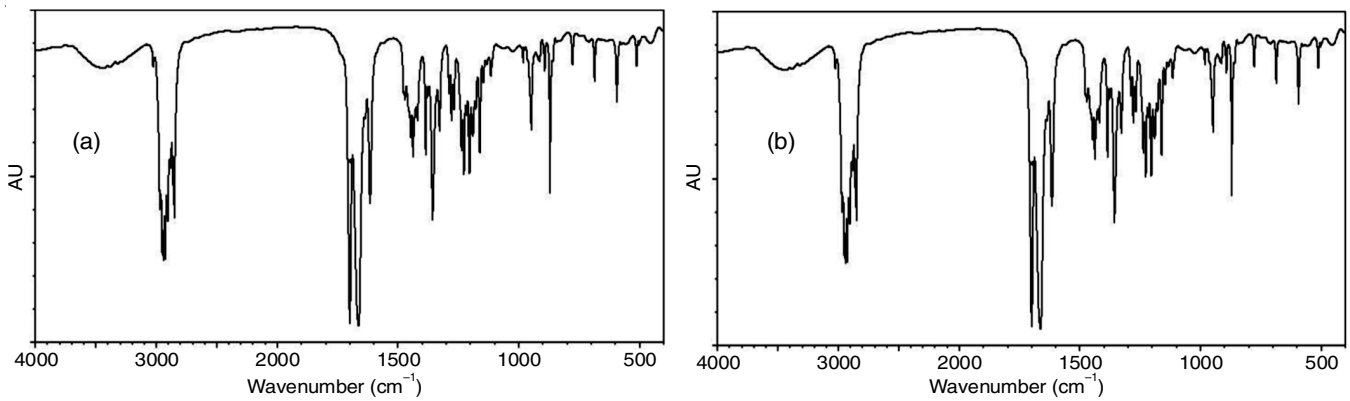


Fig. 1. FTIR spectra of (a) progesterone reference standard and (b) synthesized progesterone

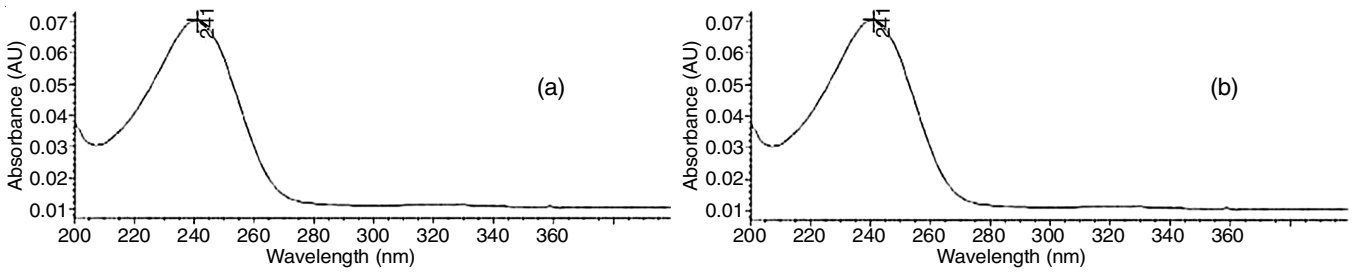


Fig. 2. UV-Vis spectra (a) progesterone reference standard and (b) synthesized progesterone

TABLE-1
IMPURITIES OR RELATIVE SUBSTANCES OF PROGESTERONE AND THEIR RETENTION TIME ON HPLC

Name	Chemical name	Relative retention time
Impurity-B	(20*S*)-20-hydroxypregn-4-en-3-one	0.59
Impurity-C	(20*R*)-20-hydroxypregn-4-en-3-one	0.93
Impurity-G	21-(cyclohexylidene)pregn-4-ene-3,20-dione	2.25

0.12%, the largest impurity (impurity-C), ranged from 0.30% to 0.33% and impurity G remained consistently at 0.08%. Cumulatively, the total impurity levels ranged from 0.50% to 0.54%, all of which met the specified criteria, with each impurity level not exceeding 0.5% and total impurities remaining below 0.8% (Table-1).

Conclusion

In summary, progesterone was synthesized from commercially available and economical 4-androstenedione *via* a three step reaction pathway, achieving an overall yield of 75% with API grade purity. The initial step involved introducing an acetenyl group to convert the C-17 ketone to a propargylic alcohol under mild conditions. This alcohol was then dehydrated to an enyne using copper sulphate as catalyst over alumina, followed by rehydration to obtain an enone intermediate. The final product progesterone was obtained with higher purity through simple recrystallization procedure. This method shows significant potential for industrial-scale progesterone synthesis due to its high yield, purity and reduced number of reaction steps.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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