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Chemical methods for the conversion of Prednisolone to 11-β-hydroxy-1,4-androstadiene-3,17-dione

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Abstract. Several microbial transformations of steroids to 17-keto cortisones through side chain cleavage have been presented in the literature; however, yields and product selectivity in these methods were low. In the present study, some new methods have been identified for the side chain cleavage of prednisolone (**1**) to form 11-β-hydroxy-1,4-androstadiene-3,17-dione (11-β-hydroxy ADD). Prednisolone upon reaction with zinc chloride in dry THF results in the formation of cleavage product in good yield (76%). 11-β-hydroxy ADD (**2**) has been formed in moderate yield (60%) under the Reformatsky reaction conditions by reacting with zincate. While performing the Wittig reaction using stable ylides, again results in the formation of compound **2** in good yield (56%). Side chain cleavage of prednisolone was confirmed from the physical and analytical data and similar when compared with the literature reports.

Keywords. Prednisolone; 11-β-hydroxy-1,4-androstadiene-3,17-dione; Chemical methods; $ZnCl_2$; Stable Wittig ylide; Reformatsky reaction

1. Introduction

Steroids play an important role in the hormonal balances. Steroids are widely used as therapeutic agents and since their inception in the market and research efforts have been made to improve production processes as well as to develop novel synthetic molecules with enhanced efficiency and reduced side effects.

Microorganisms have been widely applied for the biotransformation of steroids to generate functionalized steroids over the years.[1] These biotransformations lead to the production of steroidal contraceptive hormones and anti-inflammatory drugs from various precursors such as steroids, phytosterols, sapogenins, etc.[2] Biotransformations of steroids result in the formation of variety of synthons which can be readily used for further drug development.[3] A number of microorganisms have been utilized for steroid transformation including varieties of bacteria, fungi, yeast and algae.[4]

Among these biotransformations and microbial transformation, enzyme catalysed side chain cleavage of steroids result in the formation of 17-keto cortisones.[5,6] 17-keto cortisones have a wide range of applications including the semi-synthetic modifications for the synthesis of bioactive molecules. Androstadienedione (ADD) is one of that important 17-keto cortisones that has served as a key synthon for the development of several bioactive molecules. ADD is an important precursor for the production of sex hormones such as testosterone, estrogen, estrone and ethinyl estradiol.[3,5,6]

Androstadienedione (1,4-androstadiene-3,17-dione) is a next generation orally active prohormone. It is a direct precursor to the anabolic steroid boldenone. Boldenone is an anabolic steroid most often found in injectable form as a veterinary medicine (boldenone undecylenate). It is chemically a derivative of testosterone. Androstadienedione is used as a pharmaceutical intermediate for further synthesis of female sex hormones, in hormone replacement therapies and oral contraceptives.

Steroids like progesterone, testosteronedianabol, pregnenolone, cholesterol, sitosterol, stigmasterol, ergosterol, etc. have been reported to produce ADD through bio-transformations.[5–7] Progesterone is widely used as an important precursor of androstadienedione (ADD). Progesterone was biotransformed to ADD by *Bacillus sphaericus* with the maximum yield of 22%.[8] A major problem in the biotransformation of steroid is the poor solubility of substrates in aqueous solutions, which

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leads to extremely low productivity. Identifying the suitable microorganism is also a difficult aspect.[3b,9] Keeping in view the worldwide market for the ADD and its derivatives, several efforts were made in identifying suitable microorganisms for side chain cleavage with greater yields which remain a challenging aspect.[10–13]

To overcome these problems, several chemical methods have been developed to obtain 17-ketosteroids from the corresponding corticosteroids by cleaving the hydroxy ketone bond between C-17 and C-20.[14–16] Both the base and acid induced side chain cleavage methods have been identified. In 1981, Simons *et al*. reported the side chain cleavage of prednesolone by ethanolic KOH to afford 23% of 11-β-hydroxy-1,4-androstadiene-3, 17-dione.[14] In 2003, Pera *et al*. reported a straightforward chemical synthesis of 17-ketosteroids by cleavage of the C-17-dihydroxy acetone side chain in corticosteroids[15] by using NaOMe. However very few reports are present in the literature for the side chain cleavage of prednesolone. In 2009, Pinto *et al*. reported Bismuth (III) Triflate-Catalyzed side chain cleavage of prednesolone to 11-hydroxy ADD with 78% yield.[16]

Keeping in view the demand for ADD derivatives, alternate methods have to be explored for the bulk production of ADD derivatives. In this regard we have explored the chemical methods for the generation of ADD derivatives. During our semi-synthetic modifications on prednisolone, some chemical methods were observed for the side chain cleavage of prednisolone. In the present manuscript, we are reporting our findings for the generation of 11-hydroxy ADD through side chain cleavage by chemical methods (figure 1).

2. Experimental

2.1 *General procedure for side chain cleavage of prednesolone by $ZnCl_2$*

To the solution of prednisilone (0.1 g, 0.28 mmol) in dry THF (5 mL), dry zinc chloride (0.33 mmol) was added and refluxed under inert atmosphere for 8 h. After the completion of the reaction, monitored by TLC, the reaction mixture was quenched with excess of water and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuum to get the crude product which was purified on column to obtain the side chain cleavage product **(2)** as a colourless solid (0.63 g, 76%).

2.2 *General procedure for side chain cleavage of prednisolone by Reformatsky Reaction*

To THF (10 mL) solution of 2-bromoethylacetate (0.33 mmol), freshly activated zinc (0.42 mmol) was added under inert atmosphere and the reaction mixture was stirred for 15 h at room temperature. Reaction mixture turns viscous along the walls indicating the generation of Reformatsky reagent. Then prednisolone (0.1 g, 0.28 mmol) was added at once to the reaction mixture under inert atmosphere. Reaction mixture was continued to stir for further 24 h at room temperature. Even after 24 h the starting material remains in the reaction mixture. Reaction mixture was quenched with saturated ammonium chloride solution and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulphate and evaporated under vacuum to get the crude product which was purified on column to obtain the side chain cleavage product **(2)** as a colourless solid (0.050 g, 60%).

2.3 *General procedure for side chain cleavage of prednisolone by Wittig reaction with stable ylide*

To dry THF solution of prednisolone (0.1 g, 0.28 mmol) stable Wittig ylide (0.33 mmol) was added and the reaction mixture was refluxed for 6 h. After the completion of the reaction, monitored by TLC, reaction mixture was cooled to room temperature and evaporated under vaccum to get the impure mixture which was subjected to column chromatography to obtain the pure

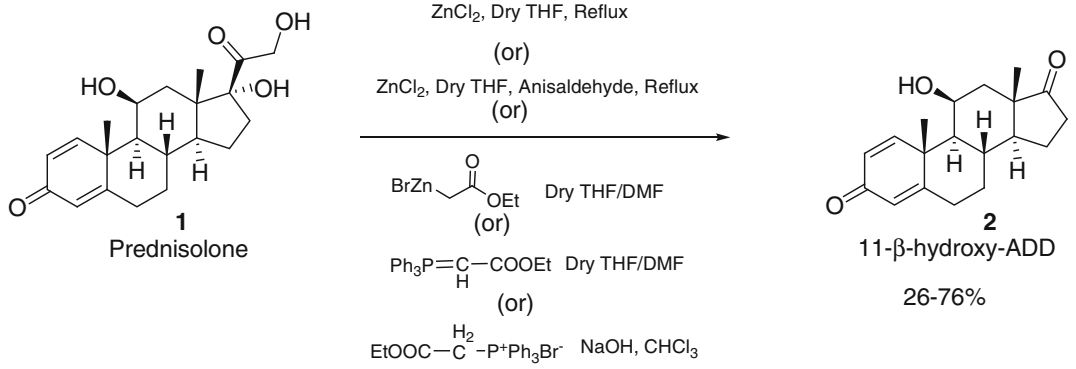


Figure 1. Chemical methods for side chain cleavage of prednisolone.

side chain cleavage product **(2)** as a colourless solid (0.047 g, 56%).

ll-β-Hydroxy-l,4-androstadiene-3,l7-dione: (M.p 184–186°C), ^{1}H NMR (400 MHz, CDCl3) δ 1.06-1.09 (dd J = 11.20 and 2.80 Hz, 1H), 1.13–1.24 (m, 5H), 1.45–1.50 (m, 4H), 1.62-1.70 (m, 2H), 1.91-2.09 (m, 3H), 2.19-2.29 (m, 2H), 2.36-2.39 (m, 1H), 2.47–2.54 (m, 1H), 2.54–2.63 (m, 1H), 4.48 (m, 1H), 6.03 (s, 1H), 6.27 (d, J = 10.00 Hz, 1H),), 7.25 (d, J = 8.40 Hz, 1H). ^{13}C NMR (100 MHz, CDCl3) δ 15.80, 21.22, 21.89, 30.87, 31.76, 32.70, 35.16, 41.00, 44.00, 46.87, 51.75, 55.90, 69.90, 122.71, 128.10, 155.67, 169.31, 186.39, 218.56. IR (Bruker alpha-FT IR spectrophotometer, Liquid, $CHCl_3$, cm^{-1}): 3010, 2964, 2940, 2916, 1736, 1660, 1622, 1447, 1407, 1162, 1051, 890, 827. MS (EI) m/z (rel intensity): 301 (M^++1), 323 (M^++23). Anal. Calcd for ($C_{19}H_{24}O_3$): C, 75.97; H, 8.05%; Found: C, 75.95; H, 8.09%.

3. Results and Discussion

When Reformatskii reaction was performed on prednisolone in order to synthesize betenolide, a serendipitous formation of 11-β-hydroxy-1,4-androstadiene-3,17-dione has been observed through the side chain cleavage. Then, Wittig reaction with a stable ylide was tried under neutral conditions to generate betenolide, again side chain cleavage was observed and the formation of 11-β-hydroxy ADD was identified (compound **2**). By using Wittig salt under basic conditions as well as under neutral conditions (stable ylide) same product formation was observed (figure 2).

While attempting the 1,3-diol protection of the side chain of prednisolone using anisaldehyde in presence of $ZnCl_2$, side chain cleavage product was observed in good yield instead of acetal. When prednisolone was treated with $ZnCl_2$ alone, again side chain cleavage happened with improved yield. Among all the methods observed, Lewis acid catalysed reaction was found to be better with 76% yield (scheme 1).

Side chain cleavage by Lewis acids can be explained by the following plausible mechanism. Addition of Lewis acid on the carbonyl oxygen followed by the concomitant cleavage of C18-C19 bond will result in the formation of compound **2** as shown in scheme 2.

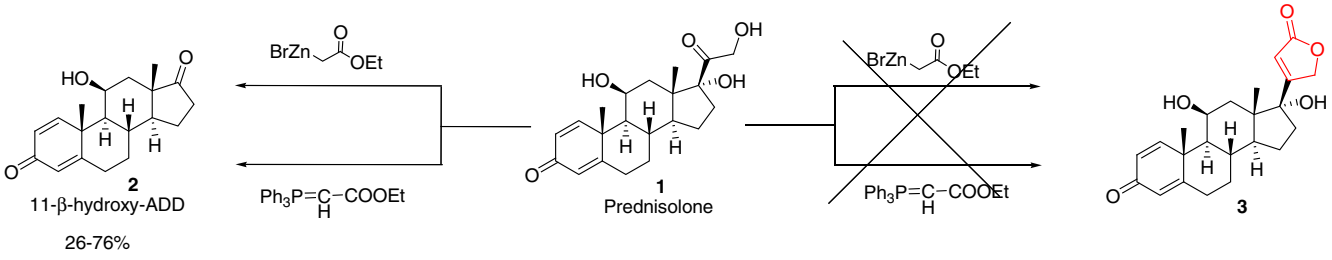
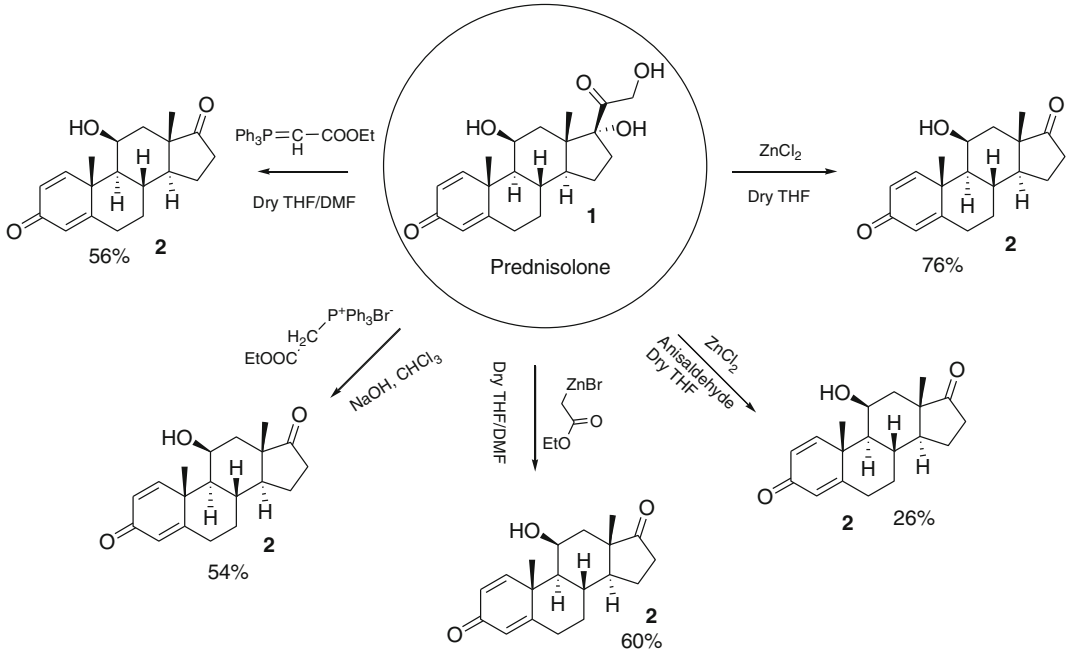
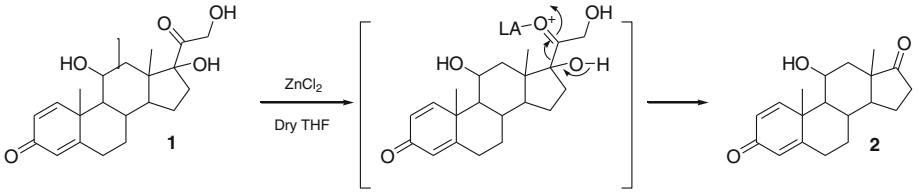


Figure 2. Attempts towards the construction of butenolide ring on prednisolone.



Scheme 1. Chemical methods for the side chain cleavage of prednisolone.



Scheme 2. Plausible mechanism for the Lewis acid catalysed side chain cleavage of prednisolone.

4. Conclusion

In conclusion, we have reported the chemical methods for the side chain cleavage of prednisolone in moderate to good yield which can be used for the further chemical transformations.

Supplementary Information

Supplementary information is available at www.ias.ac.in/chemsci.

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