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Lata Gangwar, Satish C. Pandey and Desh Deepak

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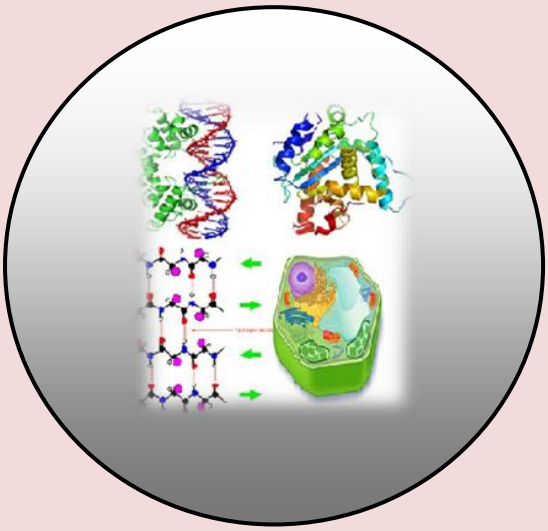

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Dr. Desh Deepak
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RESEARCH PAPER

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Synthesis of Potentially active Pregnanes with Novel D-ring and C-17 Side Chain Derivatives

Lata Gangwar, Satish C. Pandey and Desh Deepak
Department of Chemistry, University of Lucknow, Lucknow- 226007, U.P., India

ABSTRACT

Pregnanes are known to have anti-inflammatory, hypolepdemic, glucocorticoid activities. The major activities depend on the various groups present in D-ring of the steroid that may cause its binding to digitalis receptor of heart muscles and inhibit the enzymes in membranes cells and tissues. These pregnane has been synthesized by 16-dehydropregnenolone acetate (16-DPA), which was transformed by the easily available diosgenin. In the present study we have synthesized the transformations of 16-DPA into the twenty-three different derivatives of Pregnane. The structures of these synthesized pregnane derivatives were confirmed by NMR spectroscopy.

Key words: 16-DPA, Pregnane and NMR.

INTRODUCTION

Certain Pregnanes and related steroids bind to the cardiac glycoside recognition site on Na^+, K^+ ATPase and inhibit the enzyme (the sodium pump) in membranes, cells and tissues *(Templeton J F et. al., 1992)*. The 20□-hydroxy substituent can replace the □□□-unsaturated γ-lactone of the cardiac glycosides by effectively binding to the ouabain binding site of heart muscle and the C-20 alcohol can still retain positive ionotrpoic activity *(Templeton J F et. al., 1992)*. The 20□- and 20□-amino and -nitro, 20□-hydroxy and related derivatives are comparable with the corresponding 20□-hydroxy derivative of pregnane for binding potency in a [^{3}H] ouabain radiolegand assay *(Chow E et.al., 1979)*. The two most important recent developments in the field of steroids have been the discovery of the potentiation of anti-inflammatory activity by the introduction of a 16-methyl into the intact or modified hydrocortisone molecule *(Cairns J et. al., 1978)*. Alkylation at positions 16, 17 in the steroid molecule has attracted considerable attention because of the widely different effects it can have on biological activity. In progesterone a 16-methyl substituents in 9-fluoroprednisolone have the unique effect of completely counteracting the potent salt retaining effect of the 9-fluorine atom and enhancing the glucocorticoid activity *(Cairns J et.al., 1976)*. Alkylation at C-17 on the other hand, slightly increases the potency of progesterone especially with regard to the oral activity *(Cairns J et.al., 1976)*. A 17-methyl group in 11-dehydrocorticosterone acetate substantially reduces activity in the liver glycogen test and in another series of corticosterone derivatives a 17-methyl substituent resulted in a similar reduction of activity. A series of compounds, which have shown anti-inflammatory *(Cairns J et. al., 1978)*, hypolepdemic activities *(Cairns J et.al., 1976)* and these compounds have shown to bind to the digitalis receptor of heart muscles and inhibit the enzymes in membranes cells and tissues *(Cairns J et. al., 1978)*.

RESULT AND DISCUSSION

As a logical extension of these observations in this paper the combined effect of introducing alkyl substituents at the adjacent positions C-16 and C-17 and alkylation at C-20 were investigated. The most important aspect of these compounds was that the starting material of these compounds was easily available diosgenin which could be easily transformed to 16-dehydropregnenolone acetate (16-DPA) which on further transformations results into the synthesis of required compounds. The work started right from synthesis of 16-dehydropregnenolone acetate (16-DPA) (2) *(Swaminathan S et. al.,*

1987) using improved procedure for preparation of this compound by Manroe E. Wali et al. The 16-dehydropregnenolone acetate (16 DPA) (2) have double bond in conjugation with carbonyl group at C-20 in its structure which helps in introduction of ethylene glycol group at C-16 and resulting into the formation of 3β-acetoxy-16-(ethylene glycol)-5-pregnene-20-one (3) *(Engel C R et.al., 1962).* The epoxidation *(Sangwan K N, 1985)* of compound (2) was carried out by reacting it with 30% hydrogen peroxide in the presence of 6N, NaOH and resulting into 16□, 17-epoxy pregnenolone (4). 16-DPA compound (2) was treated with methyl magnesium iodide at 0^0C temperature resulting into compound 3β-acetoxy-16, 17-dimethyl-pregna-5-ene-20-one (5) [4]. 16-DPA (2) was reduced with sodium borohydride *(Hossain M et.al., 1979)* under mild conditions to afford the 20-ol i.e. 3β -acetoxy-5,16-pregna-diene-20-ol (6). Further this compound (6) was also reacted with para toluene sulphonic acid *(Benn W R et.al., 1964)* to yield compound (7) i.e. 3β,16-diacetoxy-5,17(20)-pregna-diene, which was selectively deactylated (by sodium methoxide) into 3β-hydroxy-16-acetoxy, 5,17(20)-trans pregnadiene (7A) & 3β,16-dihydroxy-5,17(20)-trans pregnadiene (7B). Further to enhance the activity of the compound (7) it was epoxidised with meta chloro per benzoic acid *(Arthur I. Vogel et.al., 1973)* resulting into epoxidation of stearicIly hindered 5,6 double bond as well as 17(20) double bond of (7) and formation of 3,16-dihydroxy-5(6),17(20)-diepoxy-pregnane (7C). (Scheme 1)

The Kishner reduction-elimination of the compound (4) afforded (Scheme 2) two isomeric unsaturated alcohols together with the steroidal pyrazole. This strategy was followed for the preparation of 5,17(20)-pregna-diene-3□□□□-diol (8) from the epoxide (4). The reaction involved refluxing of appropriate quantities of (4) and hydrazine hydrate to yield (8) *(Benn W R et.al., 1964).* The compound 3□,16-diacetoxy-5-pregnene-17-ol-20-one (9) *(Wendler N L et.al., 1960)* was also synthesized by opening the epoxide ring of compound (4). The compound (4) was treated with boron trifluoride etherate in benzene resulting into 16-fluoro-5-pregnene-3□□□□□diol-20-one (10) *(Barton D H R et.al., 1975)* as major product along with minor products. The compound (4) when subjected to methyl magnesium iodide at 0^0C temperature the 3, 20-dihydroxy-16, 17-epoxy-20methyl-pregn-5-ene compound (11) was formed. Similarly, compound 3β, 20-dihydroxy-16(17)-epoxy-20-ethyl pregn-5-ene (12) was synthesized by reacting compound (4) with ethyl magnesium bromide at 0^0C temperature *(Cairns J et.al., 1976).* The compound (4) was brominated *(Allen W S, 1960)* with 30% HBr in acetic acid resulting into formation of 17-bromo-5-pregnene-3, 16-diol (13). (Scheme 2)

Further the compound (4) 16□, 17-epoxy pregnenolone when refluxed with Grignard reagent in benzene interesting changes were observed at C-16 and C-17 positions of the epoxide. When compound (4) was treated under reflux with methyl magnesium iodide epoxide ring was opened resulting into introduction of methyl group at C-17 and hydroxy group at C-16 in the 3,16-dihydroxy-17-methyl-pregna-5-ene-20-one (14). An attractive means for the preparation of steroid hormones of the adrenocortical type advanced by Marker as a result of his finding that a 16-DPA by addition of -OH at the C-16 position in the D-ring. When 16-DPA compound (2) was treated with aluminum amalgam *(Kirk DN & Maria L et.al., 1979)* compound 3β, 16-dihydroxy-pregn-5-ene-20-one (15) was synthesized. This compound on further treatment with hydroxylamine hydrochloride and then reduction *(Templeton J F et.al., 1992)* with sodium in propanol give amine 3β,16-dihydroxy-20-amino-pregn-5-ene (15A). The mild sodium borohydride reduction *(Hossain M et.al., 1979)* of the compound (15) gave the compound 3β, 16, 20-trihydroxy-pregn-5-ene (15B). Similarly when compound (4) was treated with ethyl magnesium bromide under reflux ethyl group was introduced at C-17 and hydroxy group at C-16 in the 3β, 16-dihydroxy-17-ethyl pregn-5-ene-20-one (16). The compound (14) and compound (16) on treatment with hydroxylamine gave the oxime *(Templeton J F et.al., 1992)* and which on reduction with sodium in propanol gave corresponding amines 3, 16-dihydroxy-20-amino-17-methyl-pregna-5-ene (14A) and 3β, 16-dihydroxy-20-amino-17-ethyl pregn-5-ene (16A).

The compound (14) and compound (16) were subjected to mild sodium borohydride reduction *(Hossain M et.al., 1979)* of the carbonyl groups at C-20 and forming 3,16,20-trihydroxy-17-methyl-pregna-5-ene (14B) and 3β,16,20-trihydroxy-17-ethyl pregn-5-ene (16B) (Scheme 3).

In the present study we have emphasized on series of compounds, which have shown anti-inflammatory, hypolepdemic activities and these compounds have shown to bind to the digitalis receptor of heart muscles and inhibit the enzymes in membranes cells and tissues. The most important aspect of these compounds was that the starting material of these compounds was easily

available diosgenin which could be easily transformed to 16-dehydropregnenolone acetate (16-DPA) which on further transformations results into the synthesis of required compounds.

EXPERIMENTAL SECTION

Melting points were determined in an open capillary and are uncorrected. Optical rotations were measured on Autopol III Automatic polarimeter. ^{1}H, ^{13}C and 2D NMR spectrums were recorded on 200MHz and 300MHz (Bruker) spectrometer in $CDCl_3$ with TMS as internal standard. TLC was performed on silica-gel G (Qualigens) and silica-gel 60-120 mesh (Qualigens) was used for column chromatography.

3β-acetoxy-5,16-pregnadiene-20-one or 16 DPA (2): Diosgenin (25gm) was acetylated with acetic anhydride (130 ml). The acetylated compound was refluxed with (8.5 gm) pyridine hydrochloride for 15-16 hours. The reaction mixture was cooled to 15^0 C. Acetic acid (15 ml) was added to the reaction mixture followed by 20 ml of water. Chromic anhydride was added keeping the temperature between 15-20^0C. Then (150 ml) acetic acid was added. After keeping the reaction mixture for overnight it was treated first with 9 gm of 36 % formaldehyde and then with sodium acetate (22.75 gm). Reaction mixture was kept for 1 hour over steam bath. It was diluted with (875 ml) water and then compound was extracted with chloroform. The compound was purified by column chromatography over silica gel using hexane: ethyl acetate as solvent.

^{1}H NMR (300MHz, $CDCl_3$): δ 0.92 (s, H-18), 1.06 (s, H-19), 2.03 (s, -OAc), 2.27 (s, H-21), 4.51 (m, H-3), 5.39 (m, H-6), 6.71 (m, H-16).

^{13}C NMR (75MHz, $CDCl_3$): δ 196.8 (C-20), 170.5 (-OAc), 155.3 (C-17), 144.3 (C-16), 140.2 (C-5), 121.9 (C-6), 73.8 (C-3), 56.3 (C-14), 50.4 (C-9), 46.0 (C-13), 38.1 (C-4), 36.8 (C-1), 36.7 (C-10), 34.5 (C-12), 32.2(C-15), 31.5 (C-7), 30.1 (C-8), 27.6 (C-2), 27.1 (C-21), 21.3 (C-OAc), 20.6 (C-11), 19.1 (C-19), 15.6 (C-18).

mp 169^{0}C [Lit.: 168-169^{0}C]

Analytically Found: C, 77.49 %; H, 9.05 %. Calc. for $C_{23}H_{32}O_3$: C, 77.23 %; H, 8.99 %.

3β -hydroxy-16-(ethylene glycol)-5-pregnene-20-one (3): To a suspension16-dehydropregnelone acetate **(2)** (530 mg) in 4.0 ml of freshly distilled ethylene glycol 0.7 ml of borontrifluoride etherate was added. The reaction mixture was stirred at room temperature for 72 hours. The reaction was monitored over TLC. The dark brown coloured solution of reaction mixture was stirred into a cold sodium bicarbonate solution and then precipitate was extracted with chloroform, washed with water and dried over sodium sulphate. Extract was concentrated under reduced pressure and was chromatographed to give (215 gm) of compound **(3)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.63 (s, H-18), 1.00(s, H-19), 2.18 (s, H-21), 2.58 (d, J=6.3Hz, H-17), 3.38 (m) and δ3.52(m) for two ethylene glycol protons, 3.68 (m, H-3), 4.53 (m, H-16), 5.34 (m, H-6).

mp 136^{0}C; **$[\alpha]_D$:** -36^0;

Analytically Found: C, 73.43 %; H, 10.27 %. Calculated for $C_{24}H_{40}O_4$: C, 73.29 %; H, 10.15 %.

16□, 17-epoxy pregnenolone (4): 16-dehydropregnenolone acetate **(2)** (2.00045 gm) in methanol (85 ml) was stirred with 3ml of 30% hydrogen peroxide and then (0.7 ml) of 6N sodium hydroxide was added at 10^{0}C. The reaction mixture was allowed to continue at room temperature for 6 hours. Solvent was removed under reduced pressure. The residue was filtered, washed with ice cold water and extracted with chloroform. Finally the 1.7 gm of compound **(4)** was obtained by column chromatography.

^{1}H NMR (300MHz, $CDCl_3$): δ 0.92 (s, H-18), 1.06 (s, H-19), 2.10 (s, H-21), 3.52 (m, H-3), 3.68 (m, H-16) for 16□□proton, 5.33 (m, H-6);

^{13}C NMR (75MHz, $CDCl_3$): δ 205.0 (C-20), 141.2 (C-5), 121.1 (C-6), 71.7 (C-3), 71.1 (C-17), 60.6 (C-16), 50.4 (C-9), 45.6 (C-14), 42.2 (C-15), 41.5 (C-13), 37.1 (C-1), 36.7 (C-10), 31.6 (C-4), 31.5 (C-7), 31.4 (C-12), 29.8 (C-8), 27.5 (C-2), 26.0 (C-21), 20.5 (C-11), 19.3 (C-19), 15.2 (C-18);

mp 188^{0}C [Lit.: 188-194^{0}C];

Analytically Found: C, 76.20; H, 10.56. Calculated for $C_{23}H_{38}O_3$: C, 76.14; H, 10.39 %.

3β-acetoxy-16,17-dimethyl-pregna-5-ene-20-one (5): Grignard reagent was synthesized by taking Mg (0.036 gm) in sodium dried ether (4 ml) and methyl iodide (0.4ml). Methyl magnesium iodide (CH_3MgI) (0.036 gm, Mg) in 0.75 ml of tetrahydrofuran was taken at 0^0C in the nitrogen atmosphere. Copper (II) acetate (0.004gm) was added to it. After 10 minutes 16-dehydropregnenolone acetate **(2)** (0.075gm) was added in dry tetrahydrofuran (0.3ml). The change was observed on the TLC plate after 5 minutes. The reaction was seized by adding 7.5ml of water and 0.15 gm of ammonium chloride and extracted with chloroform and purified by column chromatography to get 0.05gm of compound **(5)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.88 (s, H-18), 1.01 (s, H-19), 1.02 (s, methyl at C-17), δ1.12 (d, J=5.7 Hz, methyl at C-16), 2.13 (s, -OAc), 2.52 (s, -$COCH_3$), 3.54 (m, H-3), 5.35 (m, H-6).

mp 173^{0}C; $[\alpha]_D$: -110^0

Analytically Found: C, 77.56 %; H, 10.51 %. Calculated for $C_{26}H_{42}O_3$: C, 77.48 %; H, 10.47 %.

3β-acetoxy-5,16-pregna-diene-20-ol (6): 3β-acetoxy-5,16-pregnadiene-20-one **(2)** (56.2 mg) was taken in anhydrous methanol (2 ml) and stirred in ice salt bath. Powdered sodium borohydride (21.85 mg) was added in portion. The reaction was monitored over TLC. After 3 hours solution was acidified with dilute acetic acid. Solvent was removed under reduced pressure and was chromatographed to give (38 mg) of compound **(6)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.92 (s, H-18), 1.06 (s, H-19), 1.30 (d, J=7.8 Hz, H-21), 2.09 (s, -OAc), 4.31(q, J=7.8Hz, H-20), 4.54 (m, H-3), 5.40 (m, H-6) and 5.75 (m, H-16);

mp 141^{0}C [Lit.: 142-143^{0}C];

Analytically Found: C, 77.05 %; H, 9.56 %. Calculated for $C_{23}H_{34}O_3$: C, 76.98 %; H, 9.46 %.

3β,16-diacetoxy-5,17(20)-pregna-diene (7): To 3β-acetoxy-5,16-pregnadiene-20-ol **(6)** (30 mg) in 0.5 ml acetic acid was added 0.08 ml of acetic anhydride and the reaction mixture was stirred for proper mixing and then 2 gm of p-toluene sulphonic acid was added. The reaction mixture was kept under nitrogen atmosphere at room temperature for 48 hours. The solution turned green, a change was also observed on the TLC plate. The reaction mixture was diluted with warm water and extracted with chloroform. Organic phase was washed with sodium bicarbonate and then with water. It was concentrated under reduced pressure and finally compound was purified by column chromatography. **^{1}H NMR (300MHz, $CDCl_3$):** δ0.92 (s, H-18), 1.06 (s, H-19), □□□□□□d□□J=7.2 Hz, H-21), □□□□□ and 2.06 for two acetyl group at C-3 and C-16, □□□□□ (m, H-3), □□□□□ (m, H-6), □□□□□ (m, H-16), □□□□□ (m, H-20);

mp 219^{0}C [Lit.: 219-222^{0}C]; **$[\alpha]_D$:** -73^0

Analytically Found: C, 74.96 %; H, 9.68 %. Calculated for $C_{26}H_{40}O_4$: C, 74.79 %; H, 9.54 %.

3β-hydroxy-16-acetoxy-5,17(20)-trans pregnadiene (7A) and 3β,16-dihydroxy-5,17(20)-trans pregnadiene (7B) : 3β,16-diacetoxy-5,17(20)-pregna-diene **(7)** was deacetylated with sodium methoxide to get the two compounds 3β-hydroxy-16-acetoxy-5,17(20)-trans pregna-diene **(7A)** and 3β,16-dihydroxy-5,17(20)-trans pregna-diene **(7B)**. The 100 mg of the compound was taken into a round bottom flask and sodium methoxide was added to it. The reaction was monitored over TLC at different intervals. After working up the compound was extracted with chloroform and purified by column chromatography.

^{1}H NMR (300MHz, $CDCl_3$) (7A): δ0.64 (s, H-18), 1.11 (s, H-19), 1.15 (d, J=6Hz, H-21), 2.02 (s, -OAc), 3.53 (m, H-3), 4.84 (m, H-20), 4.85 (m, H-16), 5.35 (m, H-6).

mp of (I) 152^{0}C; **$[\alpha]_D$:** -50^0

Anal. Found: C, 76.96; H, 10.23 Calc. for $C_{24}H_{38}O_3$ C, 76.84; H, 10.08 %.

^{1}H NMR (300MHz, $CDCl_3$) (7B): δ0.77 (s, H-18), 1.01 (s, H-19), 1.14 (d, J=6Hz, H-21), 3.52 (m, H-3), 3.74 (m, H-16), 3.79 (m, H-20), 5.36 (m, H-6).

mp of (II) 182^{0}C [Lit.: 182-188^{0}C]; **$[\alpha]_D$:** -32^0

Analytically Found: C, 79.46 %; H, 10.91 %. Calculated for $C_{22}H_{36}O_2$: C, 79.35 %; H, 10.78 %.

3,16-dihydroxy-5(6),17(20)-diepoxy-pregnane (7C): 3β,16□-diacetoxy-5,17(20)-pregna-diene **(7)** (0.03gm) was taken with 0.026 gm of meta-chloroperbenzoic acid in 0.5 ml of chloroform. Solution was stirred continuously at 0^0C for first few hours and then was kept at 0^0C for 24 hours. At the end of 24 hours only slight excess of perbenzoic was left. Excess of perbenzoic acid was shaken with an excess of 10% NaOH solution. The residual alkali was removed by washing with water, extracted

with chloroform and chloroform layer was dried with $MgSO_4$. The compound **(7C)** was obtained by performing column chromatography.

^{1}H NMR (300MHz, $CDCl_3$): δ0.58 (s, H-18), 1.06 (s, H-19), 1.13 (d, J=6Hz, H-21), 2.01 and 2.02 (s, -OAc), 3.08 (m, H-6), 3.49 (m, H-20), 4.80 (m, H-3), 4.94 (m, H-16).

^{13}C NMR (75MHz, $CDCl_3$): δ170.4 (-OAc), 170.2 (-OAc), 72.8 (C-3), 71.7 (C-6), 65.4 (C-20), 63.6 (C-17), 59.3 (C-16), 57.3 (C-9), 55.7 (C-14), 43.7 (C-8), 42.9(C-13), 39.7 (C-15), 36.9 (C-1), 35.7 (C-10), 33.1 (C-7), 28.1 (C-12), 25.8 (C-4), 24.7 (C-2), 21.3 ($C\underline{H}_3CO$), 21.1 ($C\underline{H}_3CO$), 16.0 (C-19), 12.6 (C-18)

mp 146^{0}C; $[\alpha]_D$: -55^0;

Analytically Found: C, 69.61 %; H, 8.99 %. Calculated for $C_{26}H_{40}O_6$: C, 69.48 %; H, 8.79 %.

5,17 (20)-pregna-diene-3□□□□-diol (8): 16□,17-epoxy pregnenolone **(4)** (85.75 mg) was suspended in 1.8 ml of 90% hydrazine hydrate. The reaction mixture was taken refluxed at 120-125^{0}C on sand bath for 1 hour. The reaction was cooled filtered and washed thoroughly with water. The compound was extracted with chloroform and 68 mg of pure compound was obtained by running column chromatography. This compound **(8)** was identified by acetylating both the hydroxyl group present in the molecule and then running its TLC with compound (7B) synthesized. **mp** 219^{0}C [Lit.: 219-222^{0}C].

3□,17-diacetoxy-5-pregnene-17-ol-20-one (9) : 16□,17-epoxy pregnenolone **(4)** (11.3 mg) was taken in acetic acid (0.2 ml) at 15^{0}C and then cold solution of 0.02 ml of concentrated sulphuric acid in 0.2 ml of acetic acid was added. After 6 hours at 25^{0}C, 2 ml of ice cold water was added. The compound was extracted with chloroform. The chloroform extract was washed with aqueous sodium carbonate, water and saturated sodium chloride solution. It was dried over magnesium sulphate solution. It was concentrated under reduced pressure to get the product. Finally the compound **(9)** was obtained by running column chromatography.

^{1}H NMR (300MHz, $CDCl_3$): δ1.00 (s, H-18), 1.06 (s, H-19), 2.05 (s, -2OAc), 2.16 (s, H-21), 4.60 (m, H-3), 5.31 (m, H-6), 5.6 (m, H-16).

^{13}C NMR (75MHz, $CDCl_3$): δ210.7 (C-20), 170.7 (-OAc), 170.4 (-OAc), 140.3 (C-5), 122.1 (C-6), 83.4 (C-17), 76.7 (C-16), 73.8 (C-3), 48.3 (C-9), 47.0 (C-13), 43.2 (C-14), 37.9 (C-15), 36.9 (C-4), 36.8 (C-7), 36.5 (C-12), 32.6 (C-8), 31.1 (C-1), 27.4 (C-2), 25.5 (C-21), 21.3 (-OAc), 20.5 (-OAc), 19.2 (C-11), 18.5 (C-19), 13.3 (C-18).

mp 174^{0}C; **$[\alpha]_D$:** -49^0

Analytically Found: C, 69.61%; H, 8.99%. Calculated for $C_{26}H_{40}O_6$: C, 69.52%; H, 8.83 %.

16-fluoro-5-pregnene-3□□□□□diol-20-one (10): 16□,17-epoxy pregnenolone **(4)** (25 mg) was taken with borontrifluoride etherate (0.5 ml) in benzene. The temperature was maintained at 20^{0}C. After 5 minutes the change was observed on the TLC. Compound was extracted with chloroform and chromatography with hexane and ethyl acetate in silica to get the 18 mg of the pure compound **(10)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.85 (s, H-18), 1.06 (s, H-19), 2.10 (s, H-21), 3.64 (m, H-3), 4.10 (m, H-16), □□34 (m, H-6).

mp 180^{0}C; **$[\alpha]_D$:** -33^0

Analytically Found: C, 72.21%; H, 10.28%; F, 4.97%. Calculated for $C_{23}H_{39}FO_3$: C, 72.08%; H, 10.08%; F, 4.78 %.

3,20-dihydroxy-16,17-epoxy-20-methyl-pregn-5-ene (11): Grignard reagent was synthesised by taking Mg (0.012 gm) in sodium dried ether (3 ml) and methyl iodide (0.2ml). Methyl magnesium iodide (CH_3MgI) in 0.25 ml of tetrahydrofuran was taken at 0^{0}C in nitrogen atmosphere. Copper (II) acetate (0.00125gm) was added to it. After 10 minutes 16□,17-epoxy pregnenolone **(4)** (0.025gm) in dry tetrahydrofuran (0.1ml) was added. Change was observed on the TLC plate after 5 minutes. 2.5ml of water and 0.05 gm of ammonium chloride was added and extracted with chloroform and purified by column chromatography to get 0.02124 gm of compound **(11)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.98 (s, H-18), 1.02 (s, H-19), 1.26 (s, methyl at C-20), 1.36 (s, H-21), 3.46 (s, H-16), 3.49 (m, H-3), 5.35 (m, H-6).

^{13}C NMR (75MHz, $CDCl_3$): δ140.9 (C-5), 121.2 (C-6), 74.6 (C-17), 71.6 (C-3), 70.1 (C-20), 59.1 (C-16), 50.2 (C-9), 46.7 (C-14), 42.3 (C-13), 42.2 (C-4), 37.1 (C-1), 36.6 (C-10), 33.5 (C-12), 31.4 (C-7), 29.9 (C-8), 29.6 (C-2), 28.7 (methyl at C-20), 28.3 (C-21), 26.9 (C-15), 19.2 (C-11), 16.9 (C-19), 14.0 (C-18);

mp 184^{0}C; **[α]$_D$:** -36^0
Analytically Found: C, 76.14%; H, 11.18%. Calculated for $C_{24}H_{42}O_3$: C, 76.02 %; H, 11.01 %.

3β, 20-dihydroxy-16(17)-epoxy-20-ethyl pregn-5-ene (12): Grignard reagent was synthesized by taking Mg (0.5 gm) in 20 ml of sodium dried ether and ethyl bromide (6.5gm, 4.5ml). Ethyl magnesium bromide (CH_3CH_2MgBr) in 10 ml of tetra hydrofuran was taken at 0^{0}C in nitrogen atmosphere. Copper (II) acetate (0.04685gm) was added to it. After 10 minutes 16□,17-epoxy pregnenolone **(4)** (0.8355gm) in dry tetrahydrofuran (5ml) was added. Change was observed on the TLC plate after 5 minutes. For working up the reaction water (100 ml) and ammonium chloride (1.8734 gm) was added and the product was extracted with chloroform and purified by column chromatography to get 0.258gm of compound **(12)**.
^{1}H NMR (300MHz, $CDCl_3$): δ0.86 (t, E -$CH_2C\underline{H}_3$ at C-20), 0.94 (t, Z -$CH_2C\underline{H}_3$ at C-20), 1.10 (s, H-18), 1.25 (s, H-19), 1.50 (q, -$C\underline{H}_2CH_3$ at C-20), 2.03 (s, H-21), 3.48 (m, H-3), 3.87 (m, αH-16), 4.35 (m, βH-16), 5.35 (m, H-6).
mp 191^{0}C; **[α]$_D$:** -52^0
Analytically Found: C, 76.48%; H, 11.30%. Calculated for $C_{25}H_{44}O_3$: C, 76.38%; H, 11.19 %.

17-bromo-5pregnane-3, 16-diol (13): 16□,17-epoxy pregnenolone **(4)** (0.05 gm) was taken in (0.5 ml) of acetic acid and stirred with (0.085 ml) 40% HBr in acetic acid. After 25 minutes reaction mixture was poured into 3.1 ml of cold water. The product was extracted with chloroform and finally 0.0212gm of compound **(13)** was obtained by running column chromatography.
^{1}H NMR (300MHz, $CDCl_3$): δ0.88 (s, H-18), 0.93 (s, H-19), 2.11 (s, H-21), 3.45 (m, H-3), 4.53 (m, H-16), □□□33 (m, H-6);
mp 178^{0}C; **[α]$_D$:** -3^0
Analytically Found: C, 61.31 %; H, 7.60%, Br, 19.42%. Calculated for $C_{21}H_{31}BrO_3$: C, 61.12%; H, 7.48 %, Br, 19.36 %.

3, 16-dihydroxy-17-methyl-pregna-5-ene-20-one (14): Grignard reagent as above (CH_3MgI) was treated with epoxide **(4)** (0.28875 gm) in benzene (20ml). Solvents were partially removed by distillation until a vapor temperature of 70^{0}C was reached. Then the mixture was heated under reflux for 5 hours. After cooling and treatment with aqueous ammonium chloride, the product was extracted with chloroform. The pure compound **(14)** (0.135gm) was obtained by performing column chromatography.
^{1}H NMR (300MHz, $CDCl_3$): δ0.73 (s, H-18), 1.01 (s, H-19), 1.15 (s, methyl at C-17), 2.14 (s, H-21), 3.53 (m, H-3), 5.00 (m, H-16), 5.35 (m, H-6).
mp181^{0}C; **[α]$_D$:** -10^0
Analytically Found: C, 76.34%; H, 10.945. Calculated for $C_{24}H_{41}O_3$: C, 76.23%; H, 10.79 %.

3,16-dihydroxy-20-amino-17-methyl pregna-5-ene (14A): 3,16-dihydroxy-17-methyl-pregna-5-ene-20-one **(14)** (56.2mg) was refluxed with hydroxylamine hydrochloride (200 mg), ethanol (8 ml) and pyridine in water bath for 2 hours and diluted with ice water (95 ml). Precipitate was filtered to give the trans-oxime (35 mg). TLC was carried out in the following solvent systems on silica gel (Merck type 6 OH): acetone-diethyl ether, ethyl acetate- light petroleum (35-60^{0}C). Compounds were visualized by dipping of the plates in 5% sulphuric acid-ethanol followed by heating at 120^{0}C. The crude oxime without separation of intermediates was refluxed in propane-1-ol (100 ml) and sodium (2 gms) was added in portions during 2 hours. The mixture was concentrated, diluted with water, and extracted with methylene dichloride to give the compound **(14A)** (26.3mg).

^{1}H NMR (300MHz, $CDCl_3$): 0.64 (s, H-18), 0.96 (s, H-19), 0.99 (s, -CH_3 at C-17), 1.10 (s, H-18), 1.25 (s, H-19), 1.35 (s, -CH_3 at C-17), 1.83 (d, J=12Hz, H-21), 3.54 (m, H-3), 4.24 (m, H-20), 4.34 (m, H-20), 5.34 (m, H-6), 5.41 (m, H-6).
mp 211^{0}C; **[α]$_D$:** -18^0

Analytically Found: C, 75.98 %; H, 11.37 %; N, 3.85. Calculated for $C_{23}H_{41}NO_2$: C, 75.82 %; H, 11.28 %; N, 3.78 %.

3,16,20-trihydroxy-17-methyl-pregna-5-ene(14B): 3,16-dihydroxy-17-methyl-pregna-5-ene-20-one **(14)** (56.2 mg) was taken in anhydrous methanol (2 ml) and stirred in ice salt bath. Powdered sodium borohydride (21.85 mg) was added in portion. Reaction was monitored over TLC. After 3 hours solution was acidified with dilute acetic acid. Solvent was removed under reduced pressure and was chromatographed to give (38 mg) of compound **(14B)**. **^{1}H NMR (300MHz, $CDCl_3$):** δ0.97 (s, H-18), 1.02 (s, H-19), 1.25 (s, CH_3 at C-17), 1.55 (d, J=14.1Hz, H-21), 3.47 (m, H-3), 3.49 (m, H-20), 3.52 (m, H-16), 5.35 (m, H-6).

mp 192^{0}C; **$[\alpha]_D$:** -28^0.

Analytically Found: C, 75.77 %; H, 11.06 %. Calculated for $C_{23}H_{40}O_3$: C, 75.67 %; H, 10.88 %.

3β,16-dihydroxy-pregn-5-ene-20-one (15) : 16□,17-epoxy pregnenolone **(4)** (0.90 gm) was taken in (35 ml) of ethanol 95% with aqueous 10% NaHCO3 (0.9 ml) in a round bottomed flask. Stirred at room temperature with aluminum amalgam (8 gm). The reaction was monitored on TLC. As soon as the TLC showed the complete disappearance of epoxide mixture was diluted with chloroform (100 ml). Filtered through the celite and concentrated under reduced pressure to get 0.558gm of compound **(15)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.64 (s, H-18), 1.00 (s, H-19), 2.17 (s, H-21), 2.53 (d, J=6.6 Hz, H-17), 3.49 (m, H-3), 4.79 (m, H-16), 5.33 (m, H-6).

mp 251^{0}C [Lit.: 252-255^{0}C]

Analytically Found: C, 75.98 %; H, 10.81%. Calculated for $C_{23}H_{39}O_3$: C, 75.87 %; H, 10.66 %.

3β,16-dihydroxy-20-amino-pregn-5-ene (15A) : 3β,16-dihydroxy-pregn-5-ene-20-one **(15)** (56.2 mg) was refluxed with hydroxylamine hydrochloride (200 mg) ethanol (8 ml) and pyridine in water bath for 2 hours and diluted with ice water (95 ml). Precipitate was filtered to give the trans-oxime (35 mg). TLC was carried out in the following solvent systems on silica gel (Merck type 6 OH): acetone-diethyl ether, ethyl acetate- light petroleum (35-60^{0}C). Compounds were visualized by dipping of the plates in 5% sulphuric acid-ethanol followed by heating at 120^{0}C. The crude oxime without separation of intermediates was refluxed in propane-1-ol (100 ml) and sodium (2 gms) was added in portions during 2 hours. The mixture was concentrated, diluted with water, and extracted with methylene dichloride to give 0.26gm of compound **(15A)**. **^{1}H NMR (300MHz, $CDCl_3$):** δ0.65 (s, H-18), 1.00 (s, H-19), 1.89 (s, H-21), 2.41 (m, H-17), 3.24 (m, NH_2), 3.57 (m, H-20), 3.77 (m, H-3), 4.76 (m, H-16), 5.35 (m, H-6).

mp 235^{0}C; **$[\alpha]_D$:** -23^0

Analytically Found: C, 75.98%; H, 11.25%; N, 4.01. Calculated for $C_{22}H_{39}NO_2$: C, 75.82 %; H, 11.14 %; N, 3.78 %.

3β,16,20-trihydroxy-pregn-5-ene (15B) : 3β,16-dihydroxy-pregn-5-ene-20-one **(15)** (56.2 mg) was taken in anhydrous methanol (2 ml) and stirred in ice salt bath. Powdered sodium borohydride (21.85 mg) was added in portion. Reaction was monitored over TLC. After 3 hours solution was acidified with dilute acetic acid. Solvent was removed under reduced pressure and was chromatographed to give (38 mg) of compound **(15B)**.

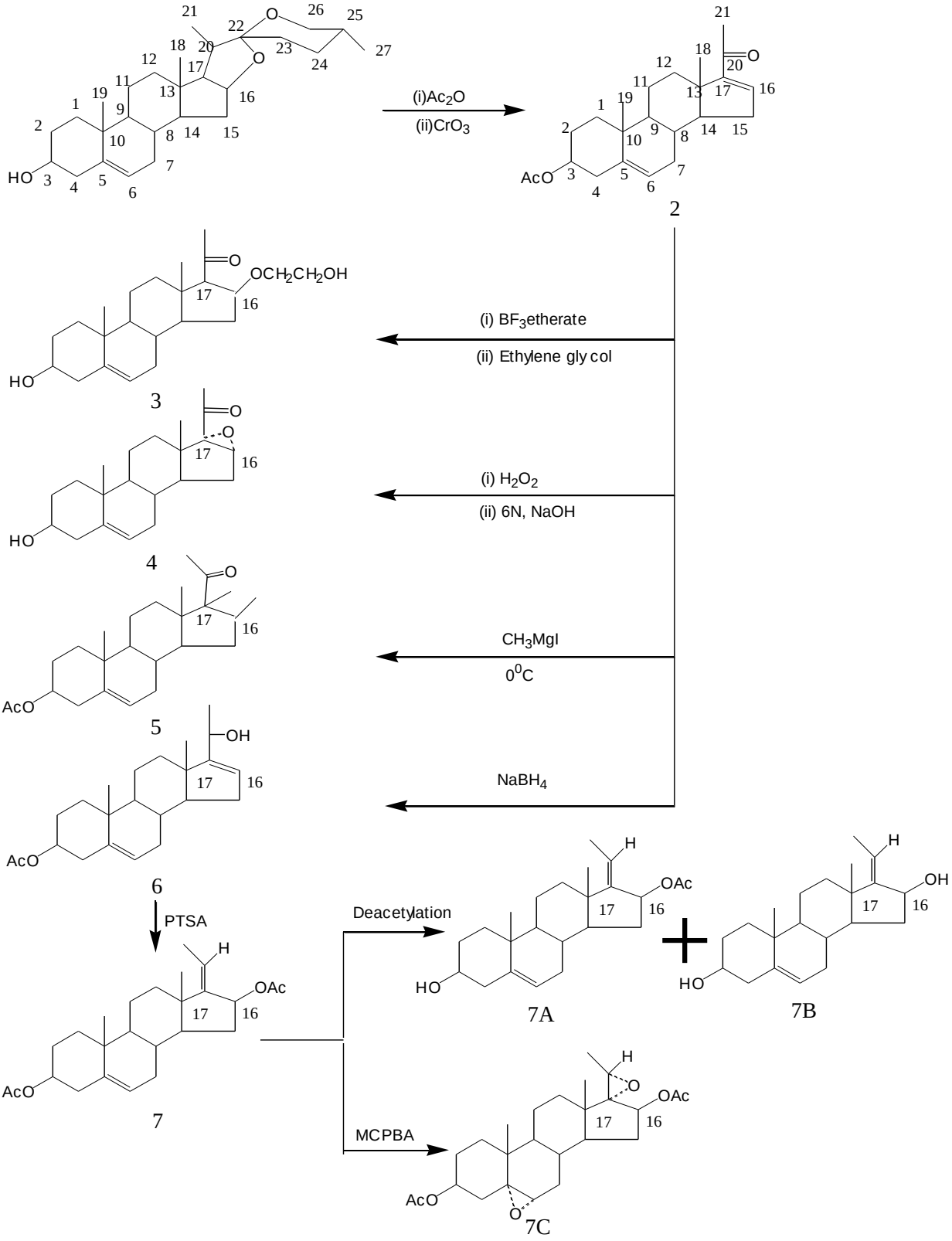
^{1}H NMR (300MHz, $CDCl_3$): δ0.66 (s, H-18), 1.00 (s, H-19), 1.99 (d, J=6.8Hz, H-21), 2.53 (m, H-17), 3.49 (m, H-3), 4.21 (m, H-20), 4.79 (m, H-16), 5.33 (m, H-6).

mp 255^{0}C; **$[\alpha]_D$:** -15^0

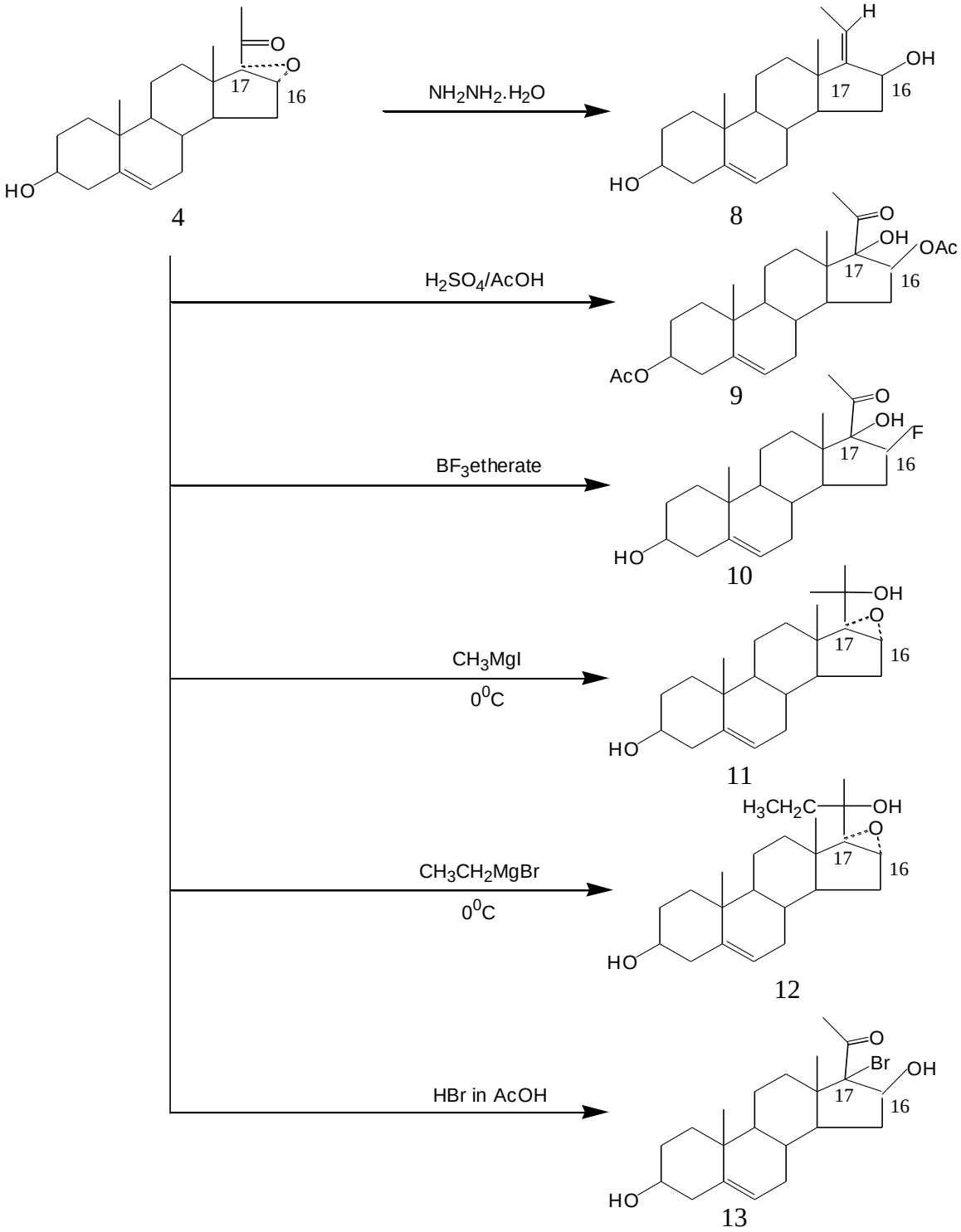
Analytically Found: C, 75.18 %; H, 10.51%. Calculated for $C_{21}H_{35}O_2$: C, 75.02 %; H, 10.38 %.

3β,16-dihydroxy-17-ethylpregn-5-ene-20-one (16): Grignard reagent was synthesized by adding magnesium (0.4 gm) in sodium dried ether (7 ml) and ethyl bromide (2.48gm) in ether (14 ml). The epoxide **(4)** (0.50125 gm) was taken into benzene (40ml). After adding Grignard reagent to the epoxide, solvents were partially removed by distillation until a vapor temperature of 70^{0}C was reached.

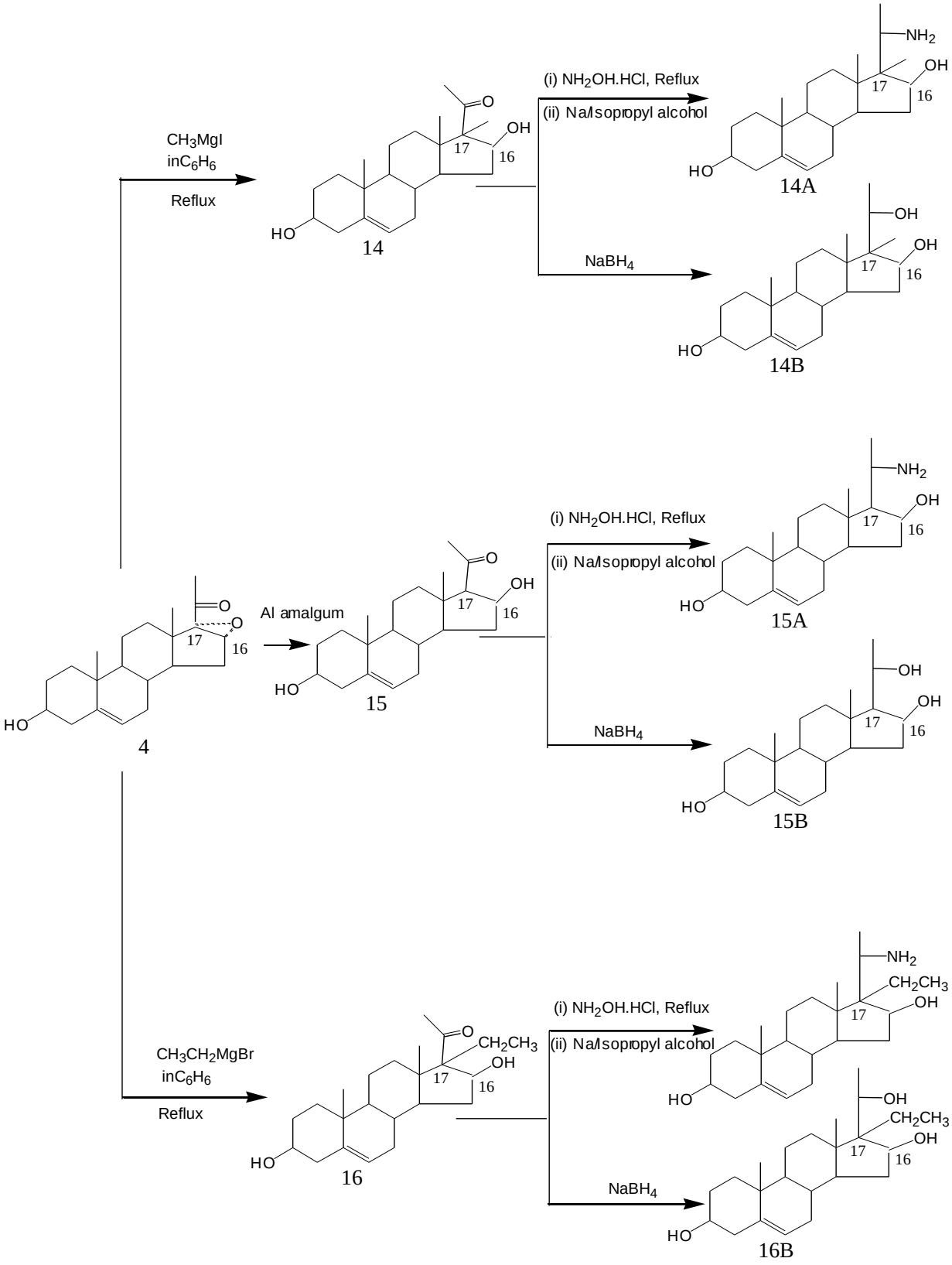
Scheme 1


(i)Ac2O
(ii)CrO3
2
(i) BF3etherate
(ii) Ethylene gly col
3
(i) H2O2
(ii) 6N, NaOH
4
CH3MgI
0 0C
5
NaBH4
6
PTSA
7
Deacetylation
7A
7B
MCPBA
7C

Scheme 2


O
O
17
16
HO
4
NH2NH2.H2O
H
OH
17
16
HO
8
H2SO4/AcOH
O
OH
OAc
17
16
AcO
9
BF3etherate
O
OH
F
17
16
HO
10
CH3MgI
0⁰C
OH
O
17
16
HO
11
CH3CH2MgBr
0⁰C
H3CH2C
OH
O
17
16
HO
12
HBr in AcOH
O
Br
OH
17
16
HO
13

Scheme 3


CH3MgI
inC6H6
Reflux
14
(i) NH2OH.HCl, Reflux
(ii) Na/Isopropyl alcohol
14A
NaBH4
14B
4
Al amalgum
15
(i) NH2OH.HCl, Reflux
(ii) Na/Isopropyl alcohol
15A
NaBH4
15B
CH3CH2MgBr
inC6H6
Reflux
16
(i) NH2OH.HCl, Reflux
(ii) Na/Isopropyl alcohol
NaBH4
16B

Then the mixture was heated under reflux for 5 hours. After cooling and treatment with aqueous ammonium chloride, the product was extracted with chloroform. The pure compound **(16)** 0.292gm was obtained by performing column chromatography.

^{1}H NMR (300MHz, $CDCl_3$): δ1.02 (s, H-18), 1.07 (t, -$CH_2\underline{C}H_3$ at C-17), 1.15 (s, H-19), 2.13 (s, H-21), 2.37 & 2.57 (m, -$C\underline{H}_2CH_3$ at C-17), 3.54 (m, H-3), 5.07 (m, H-16), 5.37 (m, H-6); **^{13}C NMR (75MHz, $CDCl_3$):** δ215.7 (C-20), 8.1 (C-17), 141.1 (C-5), 121.7 (C-6), 73.7 (C-3), 68.5 (C-16), 50.0 (C-9), 49.3 (C-14), 45.2 (-$\underline{C}H_2CH_3$ at C-17), 42.6 (C-13), 39.1 (C-4), 37.5 (C-1), 36.9 (C-10), 31.9 (C-12), 30.7 (C-12), 29.3 (C-7), 24.1 (C-8), 23.3 (C-2), 19.7 (C-21), 16.8 (C-11), 14.4 (-$CH_2\underline{C}H_3$ at C-17), 12.0 (C-19), 8.4 (C-18).
m.p.: 189^{0}C. $[\alpha]_D$: -117^0

Analytically Found: C, 76.56%; H, 10.71%. Calculated for $C_{24}H_{40}O_3$: C, 76.48 %; H, 10.64 %.

3β,16-dihydroxy-20-amino-17-ethylpregn-5-ene (16A): 3β,16-dihydroxy-17-ethyl pregn-5-ene-20-one **(16)** (56.2 mg) was refluxed with hydroxylamine hydrochloride (200 mg) ethanol (8 ml) and pyridine in water bath for 2 hours and diluted with ice water (95 ml). Precipitate was filtered to give the trans-oxime (35 mg). TLC was carried out in the following solvent systems on silica gel (Merck type 6 OH): acetone-diethyl ether, ethyl acetate- light petroleum (35-60^{0}C). Compounds were visualized by dipping of the plates in 5% sulphuric acid-ethanol followed by heating at 120^{0}C. The crude oxime without separation of intermediates was refluxed in propane-1-ol (100 ml) and sodium (2 gms) was added in portions during 2 hours. The mixture was concentrated, diluted with water, and extracted with methylene dichloride to give 21mg of compound **(16A)**.

^{1}H NMR (300MHz, $CDCl_3$): δ1.00 (s, H-18), 1.03 (t, -$CH_2\underline{C}H_3$ at C-17), 1.08 (s, H-19), 2.18 (d, J=12.9Hz, H-21), 2.28 (m, -$C\underline{H}_2CH_3$ at C-17), 3.55 (m, H-3), 4.06 (m, H-20), 4.31 (m, H-16), 5.34 (m, H-6).
mp 214^{0}C; **$[\alpha]_D$:** -10^0;

Analytically Found: C, 76.34 %; H, 11.48%; N, 3.71%. Calculated for $C_{24}H_{43}NO_2$: C, 76.25 %; H, 11.35%; N, 3.68 %.

3β,16,20-trihydroxy-17-ethyl pregn-5-ene (16B) : 3β,16-dihydroxy-17-ethyl pregn-5-ene-20-one **(16)** (56.2 mg) was taken in anhydrous methanol (2 ml) and stirred in ice salt bath. Powdered sodium borohydride (21.85 mg) was added in portion. Reaction was monitored by TLC. After 3 hours solution was acidified with dilute acetic acid. Solvent was removed under reduced pressure and was chromatographed to give (38 mg) of compound **(16B)**.

^{1}H NMR (300MHz, $CDCl_3$): δ0.86 (s, H-18), 0.98 (s, H-19), δ1.01 (t, -$CH_2\underline{C}H_3$ at C-17) 1.04 (s, H-18), 1.10 (s, H-19), 1.24 (t, -$CH_2\underline{C}H_3$ at C-17), 2.18 (d, J=6.3Hz, H-21), 2.28 (m, -$C\underline{H}_2CH_3$ at C-17), 3.54 (m, H-3), 4.06 (m, H-20), 4.31(m, H-16), 5.37 (m, H-6).
mp 193^{0}C; **$[\alpha]_D$:** -36^0

Analytically Found: C, 76.14%; H, 11.18%. Calculated for $C_{24}H_{42}O_3$: C, 76.02 %; H, 11.03 %.

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REFERENCES
Templeton J F, Ling Y, Zeglam T H, Marat K & LaBella F S (1992), *J Chem Soc Perkin Trans, I*, 2503.
Chow E, Kim R S, LaBella F S & Queen G (1979), *J Pharmacol*, 67, 345.
Cairns J, Logan R T & Woods G F (**1978)**, *J Chem Soc Perkin Trans I*, (8), 2306.
Cairns J, Hewett C L & Woods G F (**1976**), *J Chem Soc Perkin Trans I*, 1558.

Arya V P (1988), *Drugs of Future*, 13, 618**.**
Swaminathan S, Bakshi R K & Dev S (1987), *Tetrahedron*, 43, 3827.
Engel C R & Rakhit S (1962), *Canadian Journal of Chemistry*, 42, 2153.
Sangwan K N (1985), *Indian Journal of Chemistry*, 24, 639.
Hossain M & Kirk D N (1979), *Steroids*, 34(6), 677.
Benn W R & Dodson R M (1964), *J Organic Chemistry*, 29, 1142.
Arthur I. Vogel (1973), Practical Organic Chemistry**,** Third Edition,**VI**, **34/35,** 946**.**
Wendler N L & Taub D (1960), *Journal of Am Chem Soc*, 82**,** 5701.
Barton D H R (1975), *J Chem Soc Perkin Trans I*, (3), 1105.
Allen W S (1960), *Journal of Am Chem Soc*, 82, 3689.
Kirk DN & Maria L (1979), Melo S, *Steroids***,** 34(6), 683.

Corresponding author: Dr. Desh Deepak, Department of Chemistry, University of Lucknow, Lucknow- 226007, India.
Email: deshdeepakraju@rediffmail.com