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Stereoselective synthesis of (22R, 23R, 24S)-3β-Hydroxy-5-ene-22, 23-dihydroxy-24-methyl-cholestane: A Brassinolide Intermediate from 16-Dehydropregnenolone Acetate#.

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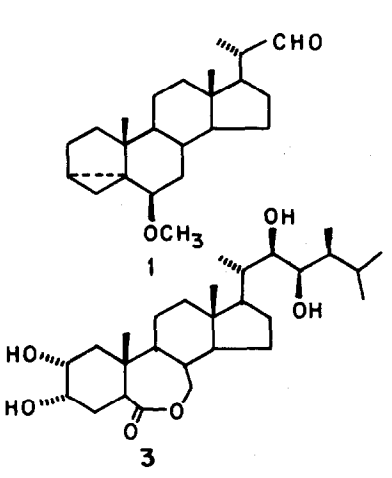
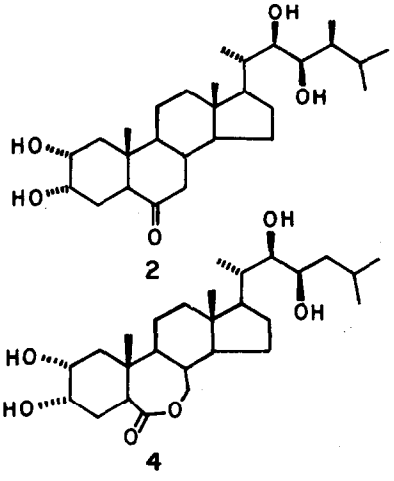
Abstract: A new synthesis of the important aldehyde 1 from easily available 16-Dehydropregnenolone acetate (16-DPA) in high yield is described. The aldehyde 1 is converted to triol 24, involving a stereoselective generation of all the four chiral centers in the brassinolide side chain. The important features of this synthesis is stereospecific generation of the acetate 14 through ene reaction using three different catalysts as well as regioselective wittig reaction on the acetoxy aldehyde 20. Conversion of triol 24 to brassinolide is known, hence this constitutes a formal total synthesis of brassinolide.

Discovery of a powerful plant growth regulator brassinolide 3 in 1979 by Grove etal[1] has led the foundation of a new era in the field of phytochemistry. Since then several other brassinosteroids have been





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isolated from natural sources such as castasterone **2** from the insect galls of Castanea spp.[2], norbrassinolide **4** from Chinese cabbage[3]. Because of their complex stereochemical structures, brassinosteroids continue to provide challenging targets for synthetic organic chemists. Specific stereochemistry in brassinolide side chain consisting of four contiguous chiral centers at C-20, C-22, C-23 and C-24 plays a crucial role in its bioactivity. The structure activity relationship studies revealed that (22R, 23R)-brassinosteriods are most active among the four possible stereoisomers of 22, 23 diols in many bioassay systems[4,5,6] and brassinolide isomer possessing 24-R configuration of the methyl group at C-24 is about one tenth as active as brassinolide. Many synthetic methods for brassinolide side chain have been widely explored[7] in the literature. In continuation of our synthetic studies of brassinosteriods we have already reported[8] a stereoselective synthesis of triol **24** starting from 3β -hydroxyandrost-5-en-17-one. Considering the scarcity and high cost of 3β-hydroxyandrost-5-en-17-one in India, we are reporting a new synthesis of aldehyde **1** starting from cheap and easily available[9] 16-dehydropregnenolone acetate **5** which in turn is converted to triol **24**. Other key features of this synthesis are a stereospecific ene reaction on compound **13** using three different catalysts and a high yield conversion of the aldehyde **1** into an appropriate Z olefin **21** by Wittig reaction on compound **20**.

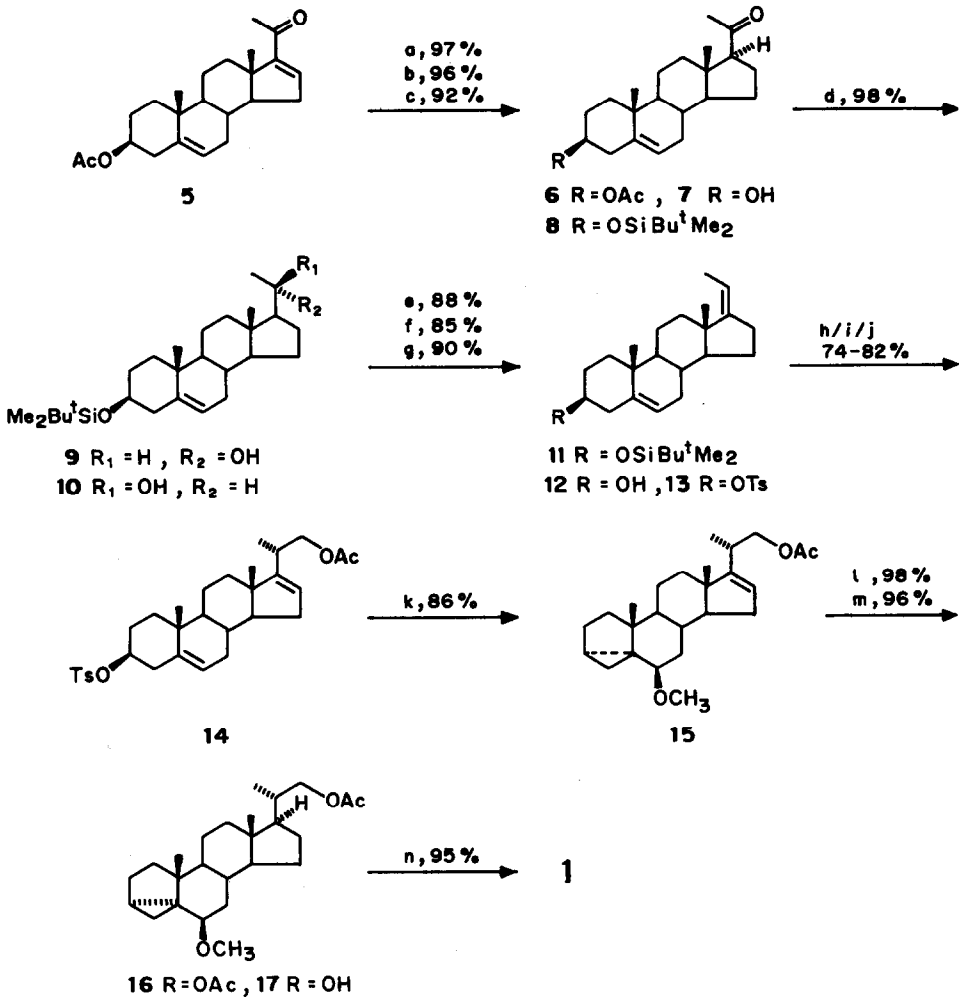
Synthesis of(20s)-3α ,5-cyclo-6β-methoxy- 5α-pregnane-20-carboxyaldehyde (1). (scheme 1)

Selective hydrogenation of the steroid **5** using 5% Pd/C in ethyl acetate afforded saturated ketone **6** in 97% yield. Hydrolysis of acetate **6** by potassium hydroxide in aq. *tert*-butanol gave **7** in 96% yield. The protection of 3β hydroxy group of **7** was carried out by *tert*-butyldimethylsilyl chloride in DMF in presence of imidazole in 92% yield. The reduction of ketone **8** with lithium aluminium hydride in THF yielded two C-20 epimeric alcohols **9** and **10** in 98% yield (20β : 20 α) = 9:1. The required C-17(20) Z olefin **11** was prepared in 88% on dehydration of tertiary alcohol **9** or **10** with phosphorus oxychloride and pyridine. The desilylation of **11** with n-tetrabutylammonium fluoride in THF furnished 3β -hydroxy (Z)-C17(20) olefin **12** in 85% yield. Tosylate **13** was prepared in 90% yield from alcohol **12** on reaction with *p*-tolunesulfonyl chloride in pyridine.The 22-acetate **14** was obtained in 82% yield by ene reaction on tosylate **13** with paraformaldehyde as an enophile, acetic anhydride and titanium triisopropoxy chloride as a lewis acid in methylene chloride. To our surprise the trimethylsilyl chloride and *tert*-butyldimethylsilyl chloride also yielded same 22 acetate in 81% and 74% respectively under similar reaction conditions. The ene reaction carried out using titanium triisopropoxy chloride, trimethylsilyl chloride and *tert*-butyldimethylsilyl chloride stereospecifically generates the natural configuration at C-20. This approach makes use of the known preference for attack on the α-face of the C-17(20) double bond and the highly ordered transition state of the ene reaction to set the stereochemistry of the C-20 carbon in the natural configuration. All the products were found to be identical in all respects with the acetate prepared according to earlier[11] method. To the best of our knowledge the use of *tert*-butyldimethylsilyl chloride and trimethylsilyl chloride as a catalyst in ene reaction has not been reported earlier. The 22-acetate **14** was converted to i-methyl ether **15** in 86% yield by refluxing with dry MeOH and pyridine, which on hydrogenation using Pd/C in ethanol gave the saturated compound **16** in 98% yield. The transfer of hydrogen from the surface of the catalyst takes place from the less hindered face of the C-16 double bond. The acetate **16** was hydrolysed by potassium hydroxide in methanol to get the C-22 alcohol **17** in 96% yield. The alcohol **17** on oxidation with pyridinium chlorochromate, potassium acetate in methylene chloride furnished the aldehyde **1** in 95% yield. This aldehyde **1** is an important intermediate[7a,c,13,16] for the synthesis of a large number of biologically active compounds including brassinolide and its analogues. The conversion of 16-dehydropregnenolone acetate **5** to the aldehyde **1** in eleven steps has been achieved in 36% overall yield. This constitutes a new synthesis of this important aldehyde **1** starting from 16-dehydropregnenolone acetate **5**.

Synthesis of (22R, 23R, 24S)-3β-hydroxy-5-ene-22, 23 dihydroxy-24-methyl-cholestane 24 (scheme 2)

The condensation of aldehyde **1** with 2-lithio 1,3 dithiane gave stereoselectively the (22R) alcohol **18** with a small amount of (22S) alcohol in 89% yield [(22R)- : (22S)- = 88:12]. The formation of (22R)-hydroxy **18** as a major product can be explained on the basis of the steric approach control as shown in scheme 2. During the attack of 2-lithio 1,3 dithiane, the path **25** involves less steric interaction hence gave predominantly (22R)

SCHEME 1



5

6 R=OAc , **7** R=OH
8 R=OSiBu^{t}Me$_2$

9 R_1=H , R_2=OH
10 R_1=OH , R_2=H

11 R=OSiBu^{t}Me$_2$
12 R=OH , **13** R=OTs

14

15

16 R=OAc , **17** R=OH

1

Reagents and Conditions: (a) H_2/pd-c, ethylacetate, 25°C, 24h; (b) KOH, t-BuOH, H_2O, 25°C, 12h, (c) t-butyldimethylchlorosilane, DMF, Imidazole, 25°C, 10h; (d) $LiAlH_4$, THF, 0°-25°C, 2h; (e) $POCl_3$, pyridine, 25°C, 30h; (f) n-Bu_4NF, THF, 25°C, 5h; (g) p-TsCl, pyridine, 25°C, 48h; (h) $Ti(i\text{-}Pr)_3Cl$, CH_2Cl_2, $(CH_2O)_n$, Ac_2O, 25°C, 48h, Ac_2O, pyridine, 24h; (i) trimethylchlorosilane, CH_2Cl_2, $(CH_2O)_n$, 25°C, 4h, Ac_2O, pyridine, 24h; (j) t-butyldimethylchlorosilane, CH_2Cl_2, $(CH_2O)_n$, Ac_2O, 25°C, 24h, Ac_2O, pyridine, 24h; (k) pyridine, dry MeOH, 70°C, 2h; (l) pd-c, EtOH, 6h; (m) KOH, MeOH, 25°C, 16h; (n) CH_3COOK, PCC, CH_2Cl_2, 25°C, 1h.

- epimer, the Cram product. In path **26** the approach of the anion is hindered by the steroidal D-ring thus resulting the anti-Cram product, (22S)- epimer was obtained as a minor product. In the ^{1}H NMR of the major (22R)-isomer the 22-H shows only a doublet with J=10 Hz indicates that it does not couple with C-20H (J=0 Hz), on the other hand in the minor isomer the 22-H shows doublet of a doublet with J=6 & 3Hz due to the coupling with C-20H and C-23H. The (22R)-hydroxy group of **18** was acetylated using acetic anhydride and pyridine to get **19** in 93% yield. The overall yield in these two steps is 83%. The compound **20** was obtained on deprotection of dithiane moiety of **19** with NBS /$BaCO_3$ in aq. Acetone in 96% yield. The Wittig reaction on **20** with triphenylphosphoniumisobutyl bromide and n-Buli in THF yielded **21** in 77% yield [(*Z*)- : (*E*)- = 86:14]. The pure *Z*-olefin was obtained after purification by column chromatography and its spectral as well as analytical data were found to be identical with the known[12] olefin. The olefin **21** was epoxidised with m-CPBA, Na_2HPO_4 in methylene chloride to obtain **22** in 95% yield. The 3,5 cyclic ring was opened with p-TSA in aq. dioxane to yield **23** in 98% yield. The epoxide **23** was opened using trimethylaluminium, n-Buli in hexane- cyclohexane to obtain **24** in 91% yield. Further elaboration of **24** to the brassinolide **3** is already well established[13], [17]. Thus the above work constitutes a formal total synthesis of brassinolide **3**.

Experimental Section:

All melting points are uncorrected. IR spectra were recorded on Perkin-Elmer spectrophotometer model 599B, using NaCl optics. ^{1}H NMR were run in $CDCl_3$ on a Brucker MSL-90 spectrophotometer and Brucker ACF-200 spectrophotometer with TMS as an internal standard. Mass specta were recorded on Finnigan Mat 1020°C mass spectrophotometer at 70ev. Elemental analyses were carried out in the analytical section of this laboratory. THF was dried over sodium benzophenone and was used freshly distilled. Tetrabutylammonium fluoride was purchased from Aldrich Chemical Company. Usual work up means, the compound was extracted with organic solvents and these extracts were washed with water, brine and finally dried over anhydrous sodium sulphate.

3β-Acetoxy-pregna-5-ene-20-one 6.

To a solution of 16-dehydropregnenolone acetate **5**, (30.6 g, 0.0859 mol) in ethyl acetate (200ml) was added 1.5g (5%) palladium on carbon catalyst. The hydrogenation was carried out using Parr apparatus at 45 psi pressure and 30°C temperature for 16h. The reaction mixture was filtered and the filtrate was dried under vacuo to obtain the saturated keto compound **6** (30 g, 98%), which was crystallised from ethyl acetate and hexane, mp.143°C (lit.[14] 147-147.5°C); IR (nujol)ν max. 1740 (O-C=O), 1720 (C=O); ^{1}H NMR(90 MHz) 0.62 (3H, s, 18-H_3), 1.0 (3H, s, 19-H_3), 2.0 (3H, s, $OCOCH_3$), 2.1 (3H, s, $COCH_3$), 3.5 (1H, m, 3-H), 5.37 (1H, dd, J=1 and 5 Hz, 6-H).

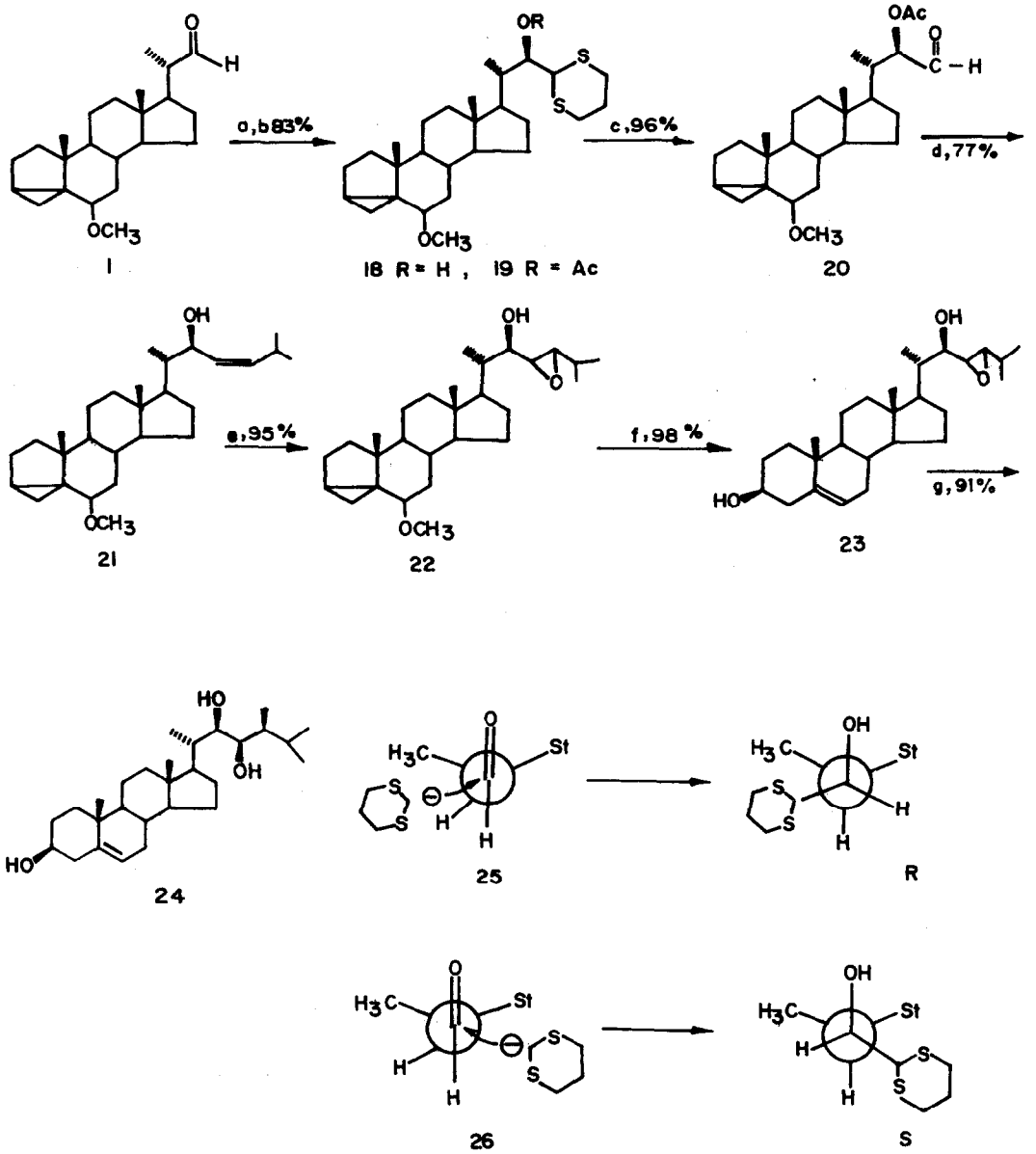
3β-Hydroxy-pregna-5-ene-20 one 7.

To a stirred solution of **6** (3.450g, 0.0096 mol) in t-butanol (50ml) was added KOH (3.1g, 0.055mol) in H_2O (5ml). The reaction mixture was stirred at 30°C for 12h, neutralised with 5% HCl solution, t-butanol was removed under vacuo and extracted with ethyl acetate. The extract on usual work up gave **7** (2.914g, 96%), which was crystallised from methanol, mp. 186°C (lit.[15] 190-191°C) ; IR(nujol)ν_{max} 1712 (C=O), 3520 (OH); ^{1}H NMR (90MHz) 0.62 (3H, s, 18-H_3), 1.0 (3H, s, 19-H_3), 2.1 (3H, s, $COCH_3$), 3.5 (1H, m, 3-H), 5.33 (1H, d, J=5Hz, 6-H).

3β-*tert*-Butyldimethylsilyloxy-pregna-5-ene-20 one 8.

tert-Butyldimethylsilyl chloride (0.3g, 2mmol) was added to a solution of pregnenolone **7** (0.316g, 1mmol) in dry DMF (5ml). Imidazole (0.272 g, 4mmol) was added to the above solution and the reaction mixture was stirred at 30°C for 10h. The reaction mixture was poured into ice and extracted with ethyl acetate. The organic

SCHEME 2



Reagents and Conditions: (a) 1,3-dithiane, n-BuLi, THF, 0°C to -20°C, 3h; (b) acetic anhydride, pyridine, 30°C, 24h; (c) NBS, $BaCO_3$, aq. acetone, 25°C, 1h; (d) $BrPh_3pCH_2CH(Me)_2$, THF, n-BuLi, 25°C, 18h; (e) m-CPBA, Na_2HPO_4, CH_2Cl_2, 25°C, 6h; (f) p-TSA, dioxane, H_2O, 60°C, 2h; (g) $(CH_3)_3Al$, n-BuLi, n-Hexane, cyclohexane, -70°C to 25°C, 20h.

layer on usual work up yielded **8** (0.398g, 92%). The product **8** was crystallised from ethyl acetate and hexane, mp.160-162°C; IR (nujol) ν max 1712 (C=O); ^{1}H NMR (200 MHz) 0.1 (6H, s, $SiMe_2$), 0.62 (3H, s, 18-H_3), 0.9 (9H, s, t-butyl CH_3), 1.05 (3H, s, 19-H_3), 2.1 (3H, s, $COCH_3$), 3.5 (1H, m ,3-H), 5.4 (1H, d, J=5Hz, 6-H); m/z 429 (M^+-1),415, 374 (100%), 355, 297, 287, 255, 239, 225, 211, 199, 185, 171, 159, 145, 133, 119, 105, 75; found C, 75.07; H, 11.00. Calc. for $C_{27}H_{46}O_2Si$ C, 75.34; H, 10.69.

3 β-*tert*-Butyldimethylsilyloxy-20(R), 20(S)-hydroxy-pregna-5-ene 9 and 10.

To a stirred solution of **8** (1.7g, 4mmol) in dry THF (20ml) was added lithium aluminium hydride (0.2g, 5mmol) at 0°C. The reaction mixture was stirred for 10 min.at 0°C and 2h at 25°C. Excess lithium aluminium hydride was decomposed by adding few drops of ethyl acetate followed by the addition of saturated NH_4Cl solution. The reaction mixture was filtered and filtrate was concentrated under vacuo. The concentrated solution was extracted with ethyl acetate which on usual work up gave **9**, and **10** and this was chromatographed to afford pure **9** and **10** (1.68, 98%). The crude product was crystallised from MeOH. From the mixture the major C-20 epimeric alcohol **9** was seperated by crystallization, mp. 145°C, the minor alcohol **10** mp. 180°C; IR (nujol) ν_{max} 3400 (OH) ; ^{1}H NMR (200MHz) 0.1 (6H, s, $SiMe_2$), 0.82 (3H, s, 18-H_3), 0.95 (9H,s, t-butyl CH_3), 1.05 (3H, s, 19-H_3), 1.22 (3H, d, J=5Hz, 21-H_3), 3.5 (1H, m, 3-H), 3.75 (1H, m, 20-H), 5.4 (1H, d, J=5Hz, 6-H); m/z 432 (M^+), 417, 375, 339, 331, 318, 299, 283, 255, 241, 235, 227, 199, 187, 173, 159, 145, 119, 105, 75 (100%); found C, 75.40; H, 11.23 ,Calc. for C, 75.10; H,11.11. The minor alcohol has ^{1}H NMR 0.73 (3H,s,18-H_3), 1.05 (3H,s,19-H_3),1.27 (3H,d, J=5Hz, 21-H_3), 3.75 (1H,m, 20-H).

3β-tert-Butyldimethylsilyloxy-(Z)-pregna-5,17 (20)-diene 11.

To a solution of alcohol **9** (0.1 g, 0.25 mmol) in dry pyridine (2ml) was added $POCl_3$ (0.5 ml, 0.19 mmol) at 0°C. The reaction mixture was stirred 10 min. at 0°C and at 25°C for 30h. The reaction mixture was poured into ice water and extracted with ethyl acetate .The extract on usual work up gave **11** (0.083 g, 88%).The product was crystallised from MeOH, mp, 145°C. The minor epimeric alcohol **10** under identical conditions also gave the same olefin **11**. The compound **11** was compared with the authentic sample prepared by silylation of hydroxy olefin **12** synthesised by known method[10] and it was found to be comparable in all respects from their mixed melting points as well as spectral data; IR (nujol) ν_{max} 1260, 1200, 980, (*Z*)- : (*E*)- = 90:10 from ^{1}H NMR.; ^{1}H NMR 0.1 (6H, s, $SiMe_2$), 0.95 (12H, s, 18-H_3, t-butyl CH_3),1.06 (3H, s, 19-H_3), 1.52 (3H, s, 21-H_3), 3.52 (1H, m, 3-H), 5.04 (1H, m, 20-H), 5.36 (1H, d, J=5Hz, 6-H); m/z 357 (M^+-57), 287, 253, 213, 171, 161, 145, 133, 121, 105, 91, 79, 75 (100%). The E olefin showed the ^{1}H NMR signals at 5.15 (1H, m) for 20-H.

3β-Hydroxy-(Z)-pregna-5,17(20)- diene 12 .

To a stirred solution of **11** (0.3 g, 0.72 mmol) in dry THF (10ml) was added tetrabutylammonium fluoride (1M solution in THF ,1.5 ml, 1.5 mmol) at 0°C. The reaction mixture was stirred 5 min. at 0°C and 2h at 25°C. THF was evaporated off and the residue was extracted with ethyl acetate, usual work up gave crude **12** (0.215 g). The crude product was column chromatographed to give pure **12** (0.184g , 85%), mp. 136-137°C (lit[10]. 136-138°C); IR (nujol) ν_{max} 3280 (OH) ,1060; ^{1}H NMR 0.88 (3H, s 18-H_3), 1.04 (3H, s, 19-H_3), 1.6 (3H, dd, J=6Hz, 21-H_3), 3.52 (1H, m, 3-H), 5.04 (1H, m, 20-H), 5.35 (1H, m, 6-H). The (E) olefin has ^{1}H-NMR signals at 5.15 (1H, m) for C-20 H.

3β-p-Toluenesulfonoxy-(Z)-pregna-5, 17(20)-diene 13.

To a stirred solution of **12** (2.53g, 8.4mmol) in dry pyridine (15ml) was added p-toluenesulfonyl chloride (3g, 15.8 mmol). Reaction mixture was kept in dark for 48h. The reaction mixture was poured in ice-cold solution

of 5% sodium bicarbonate (200ml). The compound **13** was isolated by filtration (3.1g, 82%); It was crystallised from diethyl ether and hexane, mp. 119-120°C), (lit[10]. 119-119.5°C); IR (nujol) ν_{max} 1605, 1200, 1180, 980, 960, 880 and 825; ^{1}H NMR 0.87 (3H, s, 18-H_3),0.98 (3H,s, 19-H_3), 1.65 (3H, d, J=7Hz, 21-H_3), 2.44 (3H, s, tosyl CH_3), 4.29 (1H, m, 3-H), 5.13 (1H, m, 20-H), 5.32 (1H, bd, 6-H), 7.78 (4H, AB, J=8Hz, aromatic H).

20(S)-3β-p-Toluenesulfonoxy-23,24-dinor-5,16-diene-5α- cholane-22 acetate 14.

Method A

A mixture of tosylate **13** (0.454g, 1mmol), paraformaldehyde (0.125g, 1.3 mmol), acetic anhydride (0.1ml) and titanium triisopropoxy chloride (0.115g, 0.5mmol) in dry methylene chloride (10ml) was stirred at 25°C for 48h. Methylene chloride was evaporated off and the residue was extracted with ethyl acetate. Usual work up gave an oil which was the mixture of 22 alcohol and 22 acetate. The mixture was dissolved in dry pyridine(5ml). Acetic anhydride (1ml) was added to it and it was allowed to stand at 25°C for 24h. The reaction mixture was poured into ice-cold solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer on usual work up gave **14** (0.434 g, 82%), crystallised from hexane, mp. 107 - 8°C, (lit[11]. 109-10°C); IR (neat) ν_{max} 1735(O-C=O); ^{1}H NMR 0.8 (3H, s, 18- CH_3), 0.93 (3H, s, 19-H_3), 1.04 (3H, d, J=7Hz, 21-H_3), 2.02 (3H, s, $OCOCH_3$), 2.42 (3H, s, tosyl CH_3), 3.4-4.2 (3H, m, 3-H, 22-H), 5.30 (2H, m, 6-H, 16-H), 7.29 and 7.76 (4H, AB, J=10Hz, aromatic- H).

Method B

A mixture of tosylate **13** (0.114g, 0.25 mmol), paraformaldehyde (0.030g, 0.33 mmol), trimethylsilyl chloride (0.035g, 0.32 mmol), acetic anhydride (0.1ml) in methylene chloride (20ml) was stirred at 25°C for 4h. The reaction mixture was worked up as described in method A to furnish compound **14** (0.107 g, 81%).

Method C

Tosylate **13** (0.114g, 0.25mmol), paraformaldehyde (0.056g, 0.62mmol), *tert*-butyldimethylsilyl chloride (0.077g, 0.5mmol), acetic anhydride (0.1ml) were taken in methylene chloride (20ml). The mixture was stirred at 25°C for 24h and the reaction mixture was worked up as in method A to afford **14** (0.097 g, 74%).

(20S)-3α,5-Cyclo-6β-methoxy-23,24-dinor-5α-cholane-16-ene-22-acetate 15.

To a solution of **14** (1.7g, 3.2mmol) in dry methanol (15ml), dry pyridine (1ml) was added and the reaction mixture was refluxed for 2h. Methanol was removed and the residue was extracted with ether, usual work up followed by column chromatography gave **15** as a thick oil (1.072g, 86%); IR (neat) ν_{max} 1735 (O-C=O); ^{1}H NMR 0.8 (3H, s, 18-H_3), 1.04 (3H, s, 19-H_3), 1.07 (3H, d, J=7Hz, 21-H_3), 2.02 (3H, s, $OCOCH_3$), 2.81 (1H, m, 6-H), 3.37 (3H, s, OCH_3), 3.48-4.27 (2H, m, 22-H), 5.24-5.48 (1H, m, 16-H).

(20S)-3α,5-Cyclo-6β-methoxy-23,24-dinor-cholane-22-acetate 16 .

The compound **15** (1.062 g, 2.75mmol) in ethanol (35ml) was hydrogenated in Parr hydrogenator using 10% Pd/C (0.150g) for 6h at 30 psi. The catalyst was filtered and solvent was removed under reduced pressure. The compound **16** was obtained as a thick oil, which was crystallised from ethyl acetate- hexane (1.045g, 98%), mp. 123-124°C (lit[10]. mp. 124-125°C); IR (nujol) ν_{max} 1740 (O-C=O); ^{1}H NMR 0.67 (3H, s, 18-H_3), 0.93 (3H, d, J=7Hz, 21-H_3), 0.96 (3H, s, 19-H_3), 1.98 (3H, s, $OCOCH_3$), 2.69 (1H, m, 6-H), 3.24 (3H, s, OCH_3), 3.53-4.13 (2H, m, 22-H).

(22S)-3α,5-Cyclo-6β-methoxy-23,24-dinor -5α-cholane-22-ol 17.

A solution of potassium hydroxide (0.112g, 2mmol) in methanol (10ml) was added to a solution of **16** (0.378g, 0.97mmol) in MeOH (10ml). The reaction mixture was stirred at 25°C for 16h. Methanol was removed under reduced pressure. The residue was extracted with ethyl acetate . The organic layer on usual work up gave **17** as a thick oil which was purified by column chromatography on silica gel to get **17** (0.322g, 96%); IR (neat) ν_{max} 3495 (OH), 1460, 1380, 1100 and 1020 ; ^{1}H NMR 0.84 (3H, s, 18-H_3), 1.00 (3H, d, J=6Hz, 21-H_3), 1.04 (3H, s, 19-H_3), 2.76 (1H, m, 6-H), 3.28 (3H, s, OCH_3), 3.5 (2H, d, J=6Hz, 22-H)

(20S)-3α,5-Cyclo-6β-methoxy-5α-pregnane-20-carboxyaldehyde 1.

To a stirred solution of potassium acetate (0.025g) and pyridinium chlorochromate (0.185g, 0.86mmol) in methylene chloride (5ml), a solution of **17** (0.160g, 0.46mmol) in methylene chloride (1ml) was added dropwise. The reaction mixture was stirred at 25°C for 1h. The mixture was diluted with diethyl ether, filtered and filtrate was worked up in the usual way followed by column chromatography to give **1** as a solid (0.151g, 95%); mp, 82-83°C (lit[16a]. 82-83°C); IR (neat) ν_{max} 2700, 1730 (C=O), 1100 ; ^{1}H NMR 0.76 (3H, s, 18-H3), 1.00 (3H, s, 19-H3), 1.11 (3H, d, J=7Hz, 21-H3), 2.74 (1H, m, 6-H), 3.29 (3H, s, OCH3), 9.51 (1H, d, J=3Hz, H-C=O).

22(R)-22-Hydroxy-3α,5-cyclo-6β-methoxy-24-nor-5α-cholane-23-al-trimethylene dithioacetal 18.

To a stirred solution of 1,3 -dithiane (1.5g, 12.5mmol) in dry THF (30ml) under nitrogen atmosphere at 0°C was added n-Buli (15ml, 1.4M) dropwise. The resulting solution was stirred 1h at -5° to 0°C. The solution was further cooled to -20°C and aldehyde **1** (2.2g, 6.8mmol) dissolved in THF (10ml) was injected dropwise in 10 min. and stirred for 2h. To the reaction mixture water (5ml) was added , stirred for 15 minutes and extracted with ether . The ether layer on usual work up gave **18** as a thick mass which was purified by column chromatography (2.2g, 89%). The major isomer was crystallised from ethyl acetate and hexane, mp. 163°C, IR ($CHCl_3$)ν_{max} 3460 ; ^{1}H NMR 0.3-0.6(3H, m, cyclopropyl -H) , 0.76 (3H, s, 18-H_3), 0.91 (3H, d, J= 7Hz 21-H_3), 1.00 (3H, s, 19-H_3), 2.64 (1H, t, J= 3Hz, 6-H), 2.47- 3.16 (6H, m, dithiane-H), 3.29 (3H, s, OCH_3), 3.71 (1H, d, J=8Hz, 22-H), 3.87 (1H, d, J=8Hz, 23-H) ; m/z 464 (M^+),433, 414, 326, 312, 120 ; found C, 69.92 ; H, 9.32; S, 13.68 Calc. for $C_{27}H_{44}O_2S_2$ C, 69.83; H, 9.48; S, 13.79.

22(R)-22-Acetoxy-3α-5-cyclo-6β-methoxy-24-nor-5α-cholane-23-al- trimethylene dithioacetal 19.

The product **18** (2.273g, 4.9mmol) was dissolved in dry pyridine(15 ml) and acetic anhydride (5ml) ,the mixture was kept at 25°C for 16h. The reaction mixture was poured into ice cold solution of $NaHCO_3$. The acetate **19** was filtered off. (2.35g, 93%) and was crystallised from ethyl acetate and hexane, mp. 145°C, IR (nujol) ν_{max} 1750 (O-C=O) ; ^{1}H NMR 0.3- 0.6 (3H, m, cyclopropyl-H), 0.76 (3H, s, 18-H_3), 0.96 (3H, d, 21-H_3), 1.02 (3H, s, 19-H_3), 2.36- 3.18 (6H, m, dithiane-H), 2.73 (1H, t, J=3Hz, 6-H), 3.29 (3H, s, OCH_3), 3.71 (1H, d, J=10Hz, 23-H), 5.36 (1H, d, J=10Hz, 22-H); m/z 506(M^+), 447, 415, 159, 119,59; found C, 68.71; H, 9.13; S, 12.53 Calc. for $C_{29}H_{46}O_3S_2$ C, 68.77; H, 9.09 ; S, 12.65. The 22(S)- acetate showed a ^{1}HNMR signals at 5.11 (dd, J=6 and 3 Hz) for 22-H.

22(R)-22-Acetoxy-3α-5-cyclo-6β-methoxy-24-nor-cholane-23 al 20.

To a stirred solution of **19** (0.380g, 0.75mmol) in acetone (15ml) was added $BaCO_3$ (1.78g, 9mmol) at 10°C. NBS solution in aq. acetone (20 ml) was introduced to it and the reaction mixture was stirred at 25°C for 0.5h. Excess NBS was decomposed by using sodium bisulphite solution. The mixture was filtered and filtrate was poured in excess water, extracted with ether . The ether layer on usual work up gave **20** (0.3 g, 96%), which

was crystallised from ethyl acetate and hexane, mp. 55-56°C ; IR ν_{max} 1750 (O-C=O), 1740 (H-C=O) ; ^{1}H NMR 0.3-0.6 (3H, m, cyclopropyl-H), 0.77 (3H, s, 18-H_3), O.97 (3H, d, J=7Hz, 21-H_3), 1.22 (3H, s, 19-H_3), 2.16 (3H, s,$OCOCH_3$), 2.76 (1H, t, J=3Hz, 6-H), 3.28 (3H, s, OCH_3), 5.08 (1H, d, J=2Hz, 22-H), 9.43 (1H, s, O=C-H); m/z 416 (M^+), 384, 213, 145, 105, 55; found C, 75.11; H, 9.87 Calc. for $C_{26}H_{40}O_4$ C, 75.00; H, 9.62.

22 (R)- Hydroxy -3α-5- cyclo- 6β-methoxy- 5α-cholane - 23- ene 21.

To a stirred suspension of isobutyl triphenylphosphonium bromide (1.01g, 2.5mmol), in THF (5ml) was added n-Buli (2.5ml, 1.4M) in a one lot at 0°C. Aldehyde **20** (0.208 g, 0.5mmol) in THF (5ml) was added dropwise to the above reaction mixture in 5 min. The reaction mixture was left at 25°C for 18h. THF was removed and the residue was dissolved in aq. Methanol , iodomethane (2ml) was added and the reaction mixture was stirred 2h at 25°C. MeOH was removed and the residue was poured in excess water, extracted with ethyl acetate. The organic layer on usual work up followed by column chromatography on silica gel afforded compound **21** as a thick oil (0.160g, 77%). This on tituration with MeOH solidified. The Z olefin was crystallised from methanol, mp. 42-44°C,(lit[12]. 42-45°C) ; IR (neat)ν_{max} 3450 ; ^{1}H NMR 0.3-0.6 (3H, m, cyclopropyl-H), 0.71 (3H, s, 18-H_3), 0.96 (3H, s, 19-H_3), 1.00 (6H, d, J=6Hz, 26, 27-H_3), 1.05 (3H, d, J= 7Hz, 21-H_3), 2.55 (1H, m, 25-H), 2.75 (1H, m, 6-H), 3.29 (3H, s, OCH_3), 4.51 (1H, d, J=6Hz, 22-H), 5.27 (2H, m, 23-H); 414 (M^+); found C, 87.19; H, 11.07 Calc. for $C_{28}H_{46}O_2$ C, 87.10; H, 11.18. The (22E)- olefin showed a ^{1}H NMR signals 5.67(m, 2-H) for 22-H and 23-H.

23,24-Epoxy-(22R)-22-hydroxy-3α-5-cyclo-6β-methoxy-5α-cholestane 22.

The alcohol **21** (0.750g, 1.81mmol), Na_2HPO_4 (0.86g, 6.04mmol), m-CPBA (1.5g, 8.76mmol) were taken in methylene chloride (20ml) and the reaction mixture was stirred at 25°C for 6h. The mixture was filtered and the solid was washed with CH_2Cl_2. Combined filtrate was washed with 5% NaOH and then with water till alkali free. The organic layer on usual work up followed by column chromatographic purification gave **22** as a foamy solid (0.744, 95%), was crystallised from ethyl acetate and hexane, mp. 69-70°C ; IR (nujol) ν_{max} 3530 ; ^{1}H NMR 0.3-0.6 (3H, m, cyclopropyl-H), 0.72 (3H, s, 18-H_3), 1.0 (3H, d, J=7Hz, 21-H_3), 1.02 (3H, s, 19-H_3), 1.11 (6H, d, J=7Hz, 26, 27- H_3), 2.70 (1H, dd, J=4 and 4Hz, 24-H), 2.79 (1H, t, J=2Hz, 6-H), 3.08 (1H, dd, J= 4 and 5 Hz,23-H), 3.35 (3H, s, OCH_3), 3.62 (1H, d, J=6Hz, 22-H) ; m/z 430 (M^+); found C, 77.99; H, 10.71 Calc. for $C_{28}H_{46}O_3$ C, 78.09; H, 10.77.

23,24-Epoxy-(22R)-22-hydroxy-3β-hydroxy-5α-cholestane-5-ene 23.

The mixture of alcohol **22** (0.36 g, 0.83mmol), dioxane (9ml), water (3ml), p-TSA (0.038g, 0.24mmol), was heated at 60°-65°C for 1.5h. The mixture was neutralised with $NaHCO_3$ and evaporated to dryness. The residue was extracted with CH_2Cl_2 and on usual work up gave **23** (0.344 g, 98%) , was crystallised from ethyl acetate and hexane, mp.156-157°C ; IR ν_{max} 3520 ; ^{1}H NMR 0.69 (3H, s, 18-H_3), 0.86 (3H, d, J=6Hz, 26-H3), 0.87 (3H,d, J=6Hz, 27-H_3), 0.99 (3H, s, 19-H_3), 1.08 (3H, d, J=7Hz, 21-H_3), 2.67 (1H, dd, J=4 and 5Hz, 23-H), 3.08 (1H, dd, J= 4 and 4Hz, 24-H), 3.5 (1H, m, 3-H), 3.60 (1H, d, J=7Hz, 22-H), 5.37 (1H, m, 6-H) ;m/z 416 (M^+); found C, 77.68; H, 10.57 Calc. for $C_{27}H_{44}O_3$ C, 77.83; H, 10.65.

(22R,23R,24S)-3β-Hydroxy-5-ene-22,23-dihydroxy-24-methyl-cholestane 24.

Epoxydiol **23** (0.170g, 0.4mmol) was dissolved in a mixture of cyclohexane and hexane 40 ml (1:1) by boiling and then this was cooled at -70° to - 75°C. To it was added Me_3Al (5ml, 2.0 M) in hexane followed by n-Buli (0.6 ml, 1.5 M) and the reaction mixture was stirred at -75°C for 1.5h. The reaction was further stirred at 0° to 10°C for 3h and at 25°C for 16h. The reaction mixture was cooled to -78°C and 5% HCl (5 ml) was added with syringe, stirred at 25°C for 1h, extracted with ethyl acetate , and on usual work up followed by column

chromatography gave **24** (0.160g, 91%), it was crystallised from ethyl acetate and hexane, mp. 217°C (lit[17]. 219-220 °C); IR ν_{max} 3530 ; ^{1}H NMR 0.67 (3H, s, 18-H3), 0.82 (3H, d, J=6Hz, 21-H_3), 0.87 (3H, d, J=4Hz, 24-H_3), 0.92 (3H, d, J=6Hz, 27-H_3), 0.94 (3H, d, J=6Hz, 26-H_3) , 0.98 (3H, s, 19-H_3), 3.6 (1H, m, 3-H), 5.3 (1H, m, 6-H); m/z 430(M+); found C, 77.51; H, 11.02 Calc. for $C_{28}H_{46}O_3$ C, 77.71 ; H, 11.18.

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