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Stereoselective Synthesis of a New Hexanor(C_{23}-C_{28})Castasterone-20,22-Ethyl Diether from 16-Dehydropregnenolone Acetate and its Plant Growth Promoting Activity

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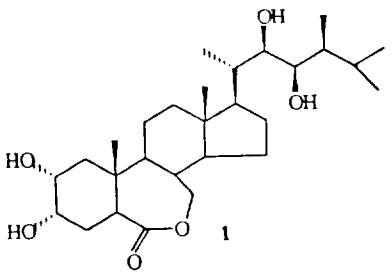
Abstract: Stereoselective synthesis of a new hexanor(C23-C28)castasterone-20,22-ethyl diether **22** has been achieved in sixteen steps from cheap and readily available 16-dehydropregnenolone acetate. This new brassinosteroid has shown typical brassin activity in mung bean epicotyl bioassay. © 1997 Published by Elsevier Science Ltd.

Brassinolide[1] **1** and its analogues, collectively known as brassinosteroids[2], are a new class of steroidal phytohormones with high growth promoting and antistress activity[3]. Since the discovery of Brassinolide **1**, more than 40 other native brassinosteroids have been isolated[4] and characterised. Recently, molecular and genetic evidence for a possible independent mechanism for auxin and brassinolide action has been found[5].



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Because of its scarcity, worldwide studies on the application of brassinosterroids for the enhancement of yield of a variety of agricultural crops and a clearly positive result[6] in field trials from India and China has intensified the research efforts towards the synthesis[7] of brassinosteroids. Owing to the structural complexities of these molecules with a polyfunctional side chain consisting of four contiguous chiral centres, most of the reported syntheses involve several steps and require exotic reagents and conditions. In order to solve this problem, several unnatural brassinosteroids of relatively lower degree of structural complexity have been designed, synthesised[8] and their plant growth promoting activity has been tested.[6b] Different qualitative structure activity relationship proposed[9] earlier indicated that the structural requirements for a brassinosteroid to be biologically active are: a A/B *trans*-ring system (5α-H), a ketone or a 7-oxa-6-keto system in ring B, *cis*-α-oriented hydroxyl groups at C-2 and C-3, *cis*-hydroxyl group at C-22 and 23 as well as methyl or ethyl substituent at C-24. But this general proposition does not hold true in case of two reports,[10,11] where the synthetic brassinosteroids lacking the required side chain in one case and having the unusual aryl substituent at C-23 in the other case showed very high brassin activity. Kerb *et al.* synthesised[10] hexanorbrassinolide-22-ethers which do not possess three asymmetric centres (C-22, C-23, C-24) of the brassinolide side chain and yet show plant growth promoting activity comparable to 28-homobrassinolide. Very recently, Ikekawa and his co workers[11] synthesised 23-phenyl brassinosteroids with plant growth promoting activity surpassing that of the parent brassinolide **1**. Brosa *et al.* in 1996 synthesised[12] four new brassinosteroids with a 2β,3β-diol and A/B *cis* and *trans* ring junction. Two of these compound with 2β,3β-diol and *A/B-cis*-ring junction elicited high activity as plant growth promoters. These authors proposed a new way to define the structural requirements for active brassinosteroids, from a detailed molecular modelling study.[12]

According to them, the activity of brassinosteroids depend on the oxygen atoms spatial situation. Therefore, the structural requirements should not be indicated as the presence or not of a specific functional group in the molecule, but as the spatial distribution of all the functionalities present in it. From all these reports and high activity of hexanorbrassinolide ethyl ether,[10] it is evident that the alkyl chain (C_{23}-C_{28}) with three contiguous chiral centers at C-22, C-23 and C-24 might not be a necessary condition for a compound to show brassin activity. These literature precedences coupled with our continuous research efforts[13] directed towards the synthesis of brassinosteroids from abundant steroidal precursors, prompted us to design new brassinosteroids, hexanor(C_{23}-C_{28})brassinolide-20,22-ethyl diether **25** and hexanor(C_{23}-C_{28})castasterone-20,22-ethyl diether **22**. These new brassinosteroids **22** and **25** are devoid of the usual six carbon (C_{23}-C_{28}) brassinolide side chain and contain a *vic*-diether functionality at C-20 and C-22.

Herein we wish to report stereoselective synthesis of a new hexanor(C_{23}-C_{28})castasterone-20,22-ethyl diether **22** from cheap and readily available 16-dehydropregnenolone acetate **2**. This hexanor brassinosteroid **22** shows considerable brassin activity in mung bean epicotial bioassay.

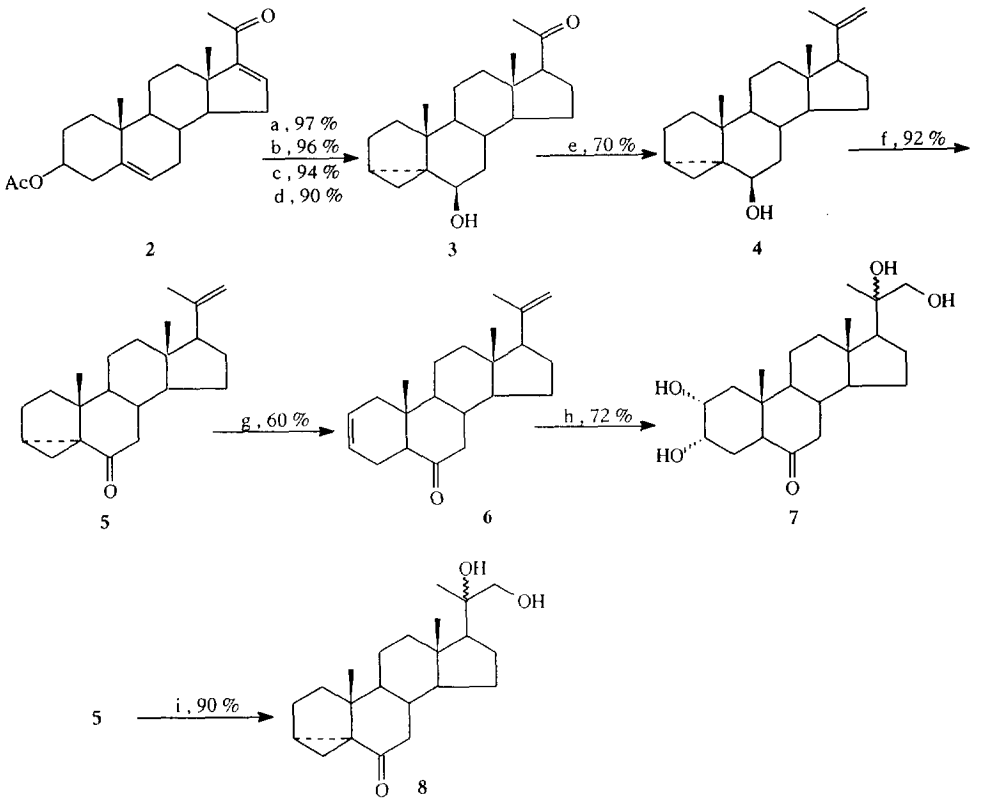
Results and Discussion

16-Dehydropregnenolone acetate **2** has been chosen as a starting material for this projected synthesis. This steroid is widely available in India. The plant dioscorea which is cultivated in many parts of India, is an abundant source of diosgenin. 16-Dehydropregnenolone acetate **2** is prepared commercially from diosgenin using Marker's procedure.[14]

The i-alcohol **3** was prepared from 16-dehydropregnenolone acetate **2** in 4 steps with an overall yield of 79% (**Scheme 1**). This transformation involves partial hydrogenation of C-16 double bond, hydrolysis of 3β-acetate functionality, tosylation of the 3β-alcohol and solvolysis of the resulting tosylate. Wittig olefination at C-20 of compound **3** afforded the 20-olefinic compound **4** (70%). Jones oxidation of the 6β-hydroxy group of compound **4** furnished the i-ketone **5**, which on acid catalysed rearrangement gave the 2,20(22)-dien-6-one **6** in 55% overall yield in two steps. *cis*-Hydroxylation of diene **6** with a catalytic amount of OsO_4 and an excess of N-methylmorpholine-N-oxide (NMO) yielded a small amount of 2,3-diol along with a number of unwanted products. Reaction of compound **6** with 2 equivalents of OsO_4 in dry benzene and catalytic amount of dry pyridine, however yielded the tetraol **7** (72%) as an epimeric mixture (1:1) at C-20. Dihydroxylation of the i-ketone **5** with one equivalent of OsO_4 in dry benzene afforded compound **8** (90%) without any enhancement in the stereoselectivity at C-20. So, our aim to generate stereoselectively the natural (20R)-configuration was not accomplished by this synthetic strategy. Moreover, use of equivalent amount of costly as well as highly toxic OsO_4 is a limiting factor in this transformation.

In order to achieve the natural (20R) configuration a different synthetic route was adopted. It was planned to introduce stereoselectively a hydroxy group at C-20 by a nucleophilic addition at the 20-ketone which could be elaborated further to our target compounds. 16-Dehydropregnenolone acetate **2** was converted to its 3β-*t*-butyldimethylsily ether **9** in three steps with 86% overall yield (**Scheme 2**). Compound **9** on treatment with 2-lithio-1,3-dithiane in THF at 0°C gave stereoselectively the (20R)-hydroxy dithaine **10** in 81% yield. Addition of 2-lithio-1,3-dithiane to 20-keto pregna derivatives is known[15] to generate stereoselectively (20R) configuration at this centre. Deprotection of the 3β-*t*-butyldimethylsilyl ether of compound **10** with tetrabutylammonium fluoride followed by removal of the dithiane moiety with $HgCl_2/HgO/CH_3CN/H_2O$ furnished the dihydroxy aldehyde **12** (75%). Tosylation of the 3β-hydroxy group of compound **12** and subsequent methanolysis of the tosylate **13** with dry MeOH and fused KOAc afforded the i-methylether **14** in 74% yield. Reduction of the C-22 aldehyde of compound **14** with $LiAlH_4$ in THF at 0°C afforded the C-20, C-22 diol **15**. The diol **15** was converted to its ethyl diether **16** with EtI in DMF using NaH as base (72%). Opening of the cyclopropane ring of **16** with catalytic amount of PTSA in aqueous dioxane gave the 3β-alcohol **17** in 84% yield. The alcohol **17** was converted to its tosyl derivative **18** (96%). Solvolysis of the tosylate **18** with KOAC/acetone/H_2O furnished the i-alcohol C-20, C-22 diether **19** which was oxidized with 8N Jones reagent to obtain the i-ketone **20** in two steps with 76% overall yield. Compound **20** was synthesised in 14 steps starting from 16-dehydropregnenolone acetate **2** in respectable

Scheme 1



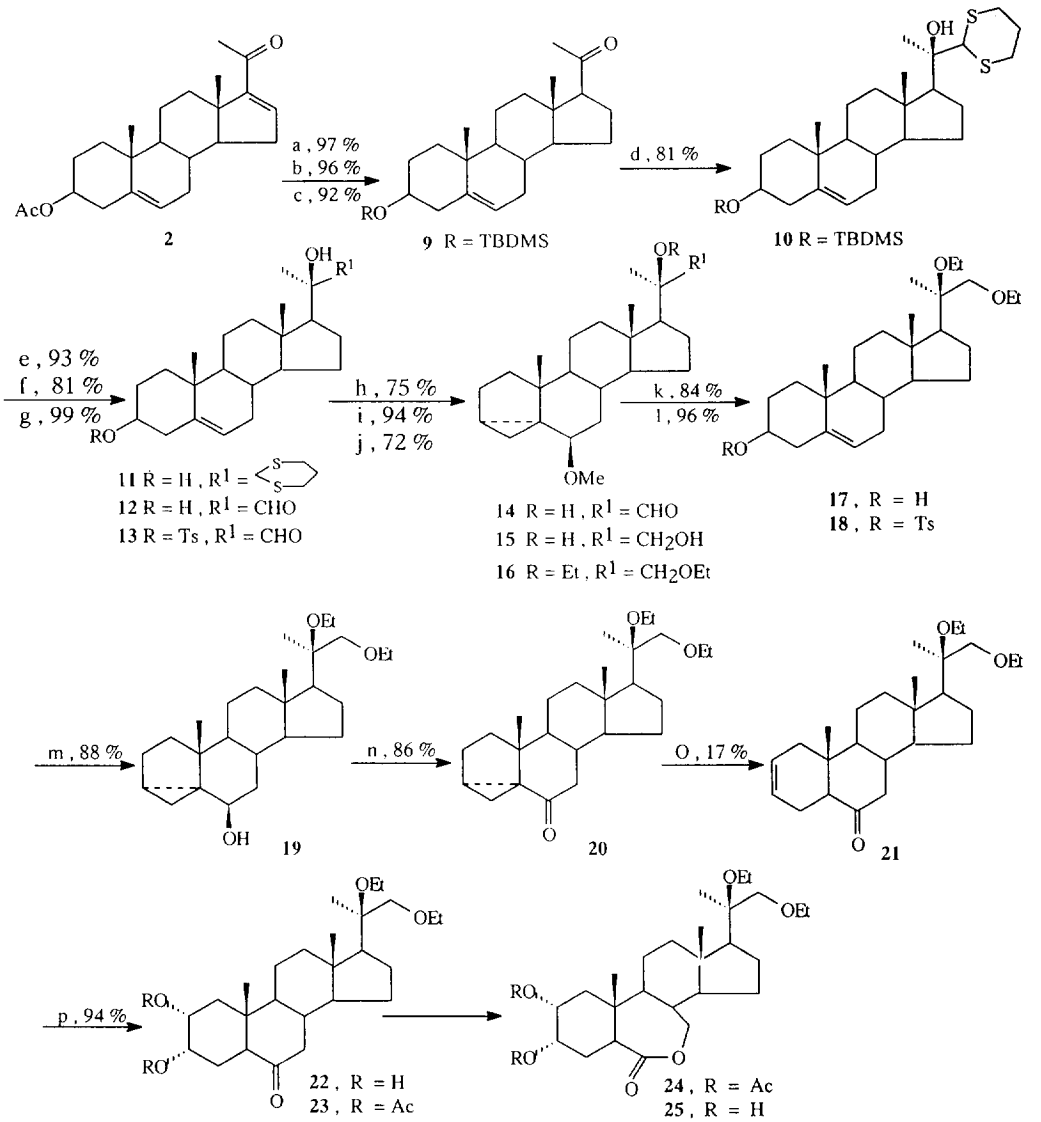
Reagents and Conditions : (a) Pd / C, H_2 , 45 psi , 30 C , 15h. (b) KOH *t* -BuOH , H_2O , 30 C ,16h ;(c) *p* -TsCl , Py , 30 C, 4h (d) CH_3COOK , acetone, H_2O, Δ , 20h ; (e) *t* -BuOK , *t* -BuOH , THF , $Ph_3P^+ CH_3I^-$, Δ , 6h ; (f) Jones reagent ,0 -5 C ,10 min (g) Pyridinium , *p* - toluene sulphonate , LiBr , DMF , 160 C , 6h ; (h) OsO_4 , Benzene , Pyridine , 30 C , 52h ; (i) OsO_4 , Benze Pyridine , 30 C , 22h

overall yield of 14.2%. But, the acid catalyzed rearrangement of the i-ketone **20** with LiBr, pyridinium *p*-toluenesulphonate in refluxing DMF for 6h gave the 2-ene-6-one **21** in poor yield (17%). Mild acid catalysed rearrangement of i-ketone **20** under a variety of conditions e.g. PTSA-sulpholane and also PTSA-dioxane-H_2O at varying temperature and reaction time failed to improve the yield of the compound **21**. In order to get the 2-ene-20,22-diether **21** in quantity from **20**, the following synthetic strategy was adopted.

Treatment of the i-ketone **20** with H_2SO_4-ACOH is expected[16] to furnish the 3β-acetoxy-6-keto-20,22-diether **26** which on alkaline hydrolysis, tosylation should afford compound **28** (**Scheme 3**). 2,3-Elimination of **28** with Li_2CO_3 in DMF should give the 2-ene-20,22-diether **21**. But, the reaction of the i-ketone **20** with H_2SO_4-ACOH at 50°C for 1h afforded the 3β-acetoxy-6-keto-22-aldehyde **29a** and **29b** as an inseparable mixture of C-20 epimers (68%) in almost 1:1 ratio. The epimeric aldehyde **29** was characterized by IR, NMR and mass spectroscopy. The expected compound **26** was not obtained in this reaction.

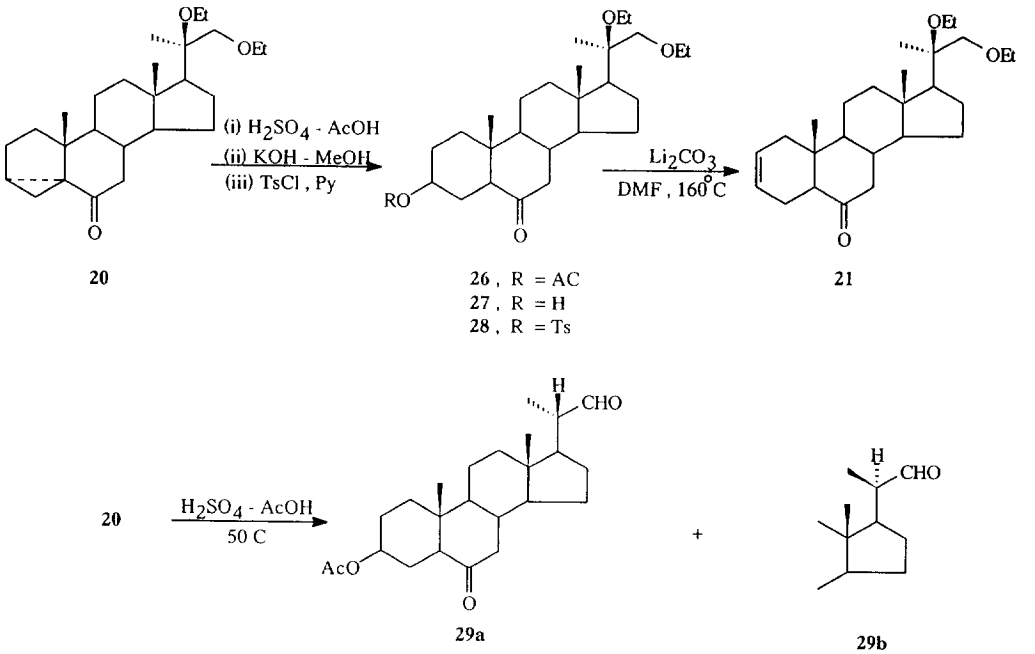
The conversion of 20,22-ethyl diether **20** to C-22 aldehyde **29** presumably takes place *via* the carbocations **30, 31** which forms the vinyl ether **32** (**Scheme 4**). This vinyl ether **32** under acidic condition furnished the epimeric aldehydes **29a** and **29b**.

Scheme 2

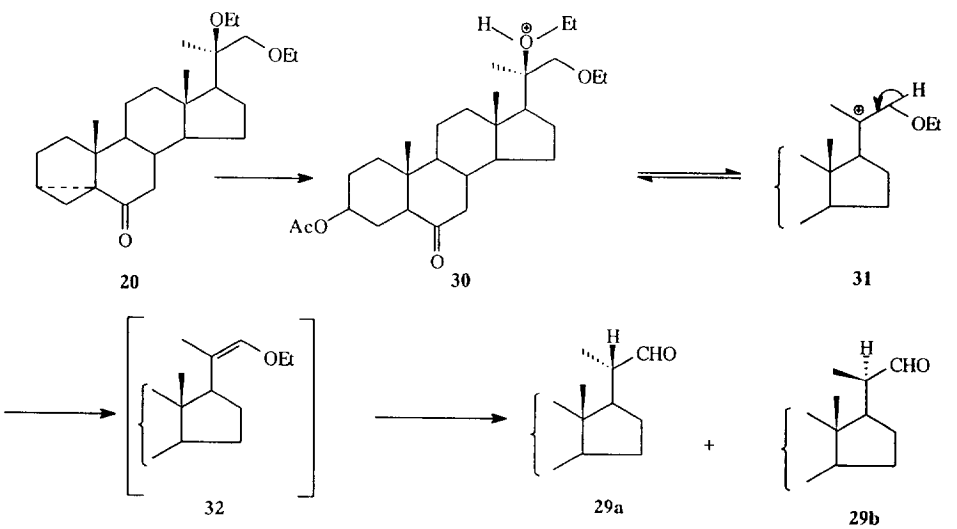


Reagents and Conditions : (a) Pd / C , H_2 , 45 psi , 30°C, 15h ; (b) KOH , *t*-BuOH , H_2O , 30°C , 16h; (c) TBDMSCl , Imidazole , DMF, 30°C, 8h ; (d) 1,3- Dithiane , n- BuLi , THF , -40°C to 0°C, 24h; (e) TBAF , THF , 30°C , 2h; (f) $HgCl_2$, HgO , CH_3CN , H_2O , Δ , 6h ; (g) TsCl , Pyridine , 30°C , 12h ; (h) CH_3COOK , MeOH , Δ , 4h; (I) $LiAlH_4$, THF , 0 to 25°C , 2h ; (j) NaH , DMF , 60°C , 2h , EtI , 30°C , 20h; (k) PTSA , Dioxane , H_2O , 50 to 60°C , 4h ; (l) TsCl , Pyridine , 30°C , 12h ; (m) CH_3COOk , Acetone , H_2O , Δ , 20h : (n) 8N Jones reagent , 0 to 5°C , 15 min ; (o) LiBr , PPTS , DMF , 150 to 160°C , 4h ; (p) OsO_4 , NMO , *t*- BuOH , H_2O , Acetone , 30°C , 4h.

Scheme 3



Scheme 4



Compound **21**, obtained in poor yield from the acid catalyzed rearrangement of **20** was osmylated with catalytic amount of OsO_4 and N-methylmorpholine-N-oxide to furnish the hexanor (C_{23}-C_{28})castasterone-20,22 -ethyl diether **22** in 94% yield (**Scheme 2**). Compound **22** was characterized by IR, ^{1}H NMR, mass spectroscopy and elemental analysis. This is a new hexanor castasterone analogue and has been synthesized in 16 steps from 16-dehydropregnenolone acetate.

The biological activity of the new brassinosteroid **22** was determined using sensitive mung bean epicotyl growth bioassay developed by Mandava and his co workers[17] with some modifications for this purpose. Parallel experiments with known (22S,23S)-28-homobrassinolide [(22S,23S)-HBR] was also carried out for comparison purpose. The data on the growth of mung bean epicotyls as influenced by (22S,23S)-HBR and the new brassinosteroid **22** are presented in **Table 1**.

Table 1:Effect of (22S, 23S)-HBR and the new brassinosteroid **22** on epicotyl length of mung bean cuttings.

Brassinosteroid Treatments	Percent increase in epicotyl length over control					
	Days after Brassinosteroid treatments					
	2	3	4	5	6	14
(22S,23S)-HBR (10^{-6}M)	165	173	126	105	96	68
New Brassinosteroid **22** (10^{-6}M)	19	30	20	15	13	11
New Brassinosteroid **22** (10^{-5}M)	38	54	43	36	33	20

This data indicate that compound **22** shows considerable growth promoting activity over control at 10^{-5}M to 10^{-6}M concentration. When used in 10-fold excess [10^{-5}M of **22** *vs* 10^{-6}M of (22S,23S)-HBR] the new brassinosteroid **22** showed 30 to 40% activity of (22S,23S)-HBR, though at the same concentration range (10^{-6}M) (22S,23S)-HBR is 6 to 9 times more active than compound **22**. It is also interesting that in both the cases there is a gradual decline in activity after three days indicating the same metabolic rates for (22S,23S)-28-homobrassinolide and the newly synthesised brassinosteroid **22**.

Experimental

All m.p.s are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrophotometer model 599B using NaCl optics. ^{1}H NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrophotometer using TMS as an internal standard and chemical shifts are given in δ (ppm). Mass spectra were recorded on a Finnigan Mat 1020C mass spectrophotometer at 70ev. All optical rotations were measured on JASCO-181 digital polarimeter using sodium light (4893A°) source. Concentrations are expressed in g/100ml of solution. Unless otherwise indicated, all solvents and reagents used were of commercial grade. Pet-ether refers to the fraction boiling between 60-80°C. Dry THF was freshly obtained by taking the liquid to reflux under nitrogen, in a recirculation still over sodium. Pyridine was purified by distillation and stored over KOH pellets. Reactions were monitored by TLC using TLC aluminium sheets, silica gel 60 F_{254} precoated, Merck, Germany and locating the spots spraying with ethanolic solution of phosphomolybdic acid followed by heating. Usual work up means the organic extract was thoroughly washed with water and brine and finally dried over anhydrous sodium sulphate. The mung bean [*Vigna radiata (L) Wilczek*] epicotyl bioassay was carried out in a plant growth room maintained at 25°C, on a 12h photoperiod with a light intensity of 2400 lux provided by cool white fluoroscent tubes and incandescent bulbs and ambient relative humidity. There were four treatments, namely, control, 10^{-6}M (22S,23S)-28-homorsassinolide [(22S,23S)-HBR], 10^{-6}M new brassinosteroid **22** and 10^{-5} M of **22**. Each experiment was tested using a total of 20 seedlings. The mean increases (average of 20 seedlings) in epicotyl length were calculated for all the treatments and compared to that of the control. An aqueous solution of (22S,23S)-HBR was prepared by dissolving the compound in small amount of ethanol and emulsified with the surfactant Tween 20.

3α,5-Cyclo-6β-hydroxypregnane-20-one (3)

Pregnenolone, prepared[13d] from 16-dehydropregnenolone acetate **2** was tosylated with *p*-TsCl in pyridine to afford its 3β-tosylate in 94% yield, m.p. 128°C; IR (nujol) ν_{max} 1710 (-C=O)cm^{-1}; ^{1}H-NMR δ 0.62 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 2.08 (s, 3H, $COCH_3$), 2.42 (s, 3H, tosylmethyl), 4.35 (m, 1H, 3-H), 5.27 (m, 1H, 6-H), 7.24-7.8 (AB quartet, J=9Hz, aromatic-H); m/z 299 (M^+-OTs), 283, 265, 255, 240, 227, 214, 199, 177, 160, 147, 129, 121, 107, 91(100%). This tosylate (3.5g, 7.4mmol) was dissolved in aqueous acetone (60ml) and to it was added fused potassium acetate (4.292g, 44mmol). The reaction mixture was refluxed on oil bath for 20h. Acetone was removed under vacuo and the residue was extracted with ethyl acetate (4x50 ml). Usual work up afforded (2.347g, 99%) of i-alcohol **3**, m.p. 182°C (hexane-ethyl acetate); IR (nujol) ν_{max} 3520 (-OH), 1710 (-C=O) cm^{-1}; ^{1}H-NMR δ 0.24-0.49 (m, 3H, cyclopropyl-H), 0.68 (s, 3H, 18-H_3), 1.18 (s, 3H, 19-H_3), 2.1 (s, 3H, $COCH_3$), 3.24 (t, 1H, J=5Hz, 6-H); m/z 316 (M^+), 298, 302, 283, 275, 261, 255, 213, 159, 145, 133, 121, 105, 91(100%); Calc. for $C_{21}H_{32}O_2$ C, 79.74; H, 10.12. Found: C, 79.64; H, 10.22%; $[\alpha]_D^{25}$+107.4° (C 2.4, $CHCl_3$).

3α,5-Cyclo-6β-hydroxypregna-20(22)-ene (4)

To a solution of *t*-BuOK (2.12g, 19mmol), prepared by the addition of metallic K (0.740g, 19mmol) in dry *t*-BuOH (10ml), was added triphenylphosphoniummethyl iodide (5.3g, 13mmol) in dry THF (50ml). The suspension showed deep yellow colour. The mixture was then stirred for 45 min and ketone **3** (2g, 6mmol) in dry THF (30ml) was added to this at 25°C followed by reflux for 6h. Then the reaction mixture was poured into a mixture of methanol and water (1:1) and the product was isolated by extraction with ethyl acetate (4x50ml). The crude material (4g) obtained by usual work up was dissolved in methanol and to it methyl iodide (4ml) was added and stirred for 2h at 30°C. Removal of methanol gave a mixture containing triphenylphosphine oxide, triphenyphlphosphine and the olefin **4**. This mixture was further purified by column chromatography over neutral alumina to obtain pure olefin **4** (1.4g, 70%) as a gum, IR (nujol) ν_{max} 3450 (-OH), 1655cm^{-1}; ^{1}H-NMR δ 0.22-0.34 (m, cyclopropyl-H), 0.62 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 1.76 (s, 3H, 21-H_3), 3.24 (t, J=5Hz, 1H, 6-H), 4.64 and 4.8 (two bs, 2H, 22-H); m/z 314 (M^+), 296, 287, 259, 253, 245, 227, 199, 185, 171, 159, 145, 131, 121, 107, 91 (100%).

3α,5-Cyclopregna-20(22)-en-6-one (5)

To a solution of olefinic alcohol **4** (0.470g, 1.5mmol) in acetone (30ml) was added Jones reagent (2ml, 8N solution) dropwise at 0°C in 2 min. The resultant solution was stirred at 0°-5°C for 10 min and then excess reagent was destroyed with methanol and the solvent was removed to obtain a gummy residue. It was extracted with ethyl acetate (3x50ml). Usual work up followed by removal of solvent under vacuo and column chromotography purification afforded the product **5** (0.454g, 97%); m.p. 106-108°C (MeOH); IR (nujol)ν_{max} 1710 (-C=O), 1645 cm^{-1}; ^{1}H-NMR δ 0.63 (s, 3H, 18-H_3), 1.0 (s, 3H, 19-H_3), 1.78 (s, 3H, 21-H_3), 4.64 and 4.8 (two bs, 2H, 22-H); m/z 312 (M^+), 297, 269, 256, 243, 229, 215, 201, 187, 173, 161, 145, 133, 120, 105, 91, 79(100%); $[\alpha]_D^{25}$ +27.6° (C 1.02, $CHCl_3$); Calc. for $C_{22}H_{32}O$ C, 84.61; H, 10.25. Found: C, 84.38; H, 10.34%.

Pregna-2,20(22)-dien-6-one (6)

To a magnetically stirred solution of *i*-ketone **5** (0.196g, 0.628mmol) in dry DMF (4ml) was added lithium bromide (0.027g, 0.31mmol) and pyridinium *p*-toluenesulfonate (0.027g, 0.1mmol). The reaction mixture was refluxed at 175°C for 3h, cooled, poured into ice water and extracted with ethyl acetate (3x25ml). Usual work up followed by evaporation of solvent yielded a mixture containing pregna-2,20(22)-dien-6-one **6** as a major product. Column chromatographic purification gave (0.118g, 60%) of pure pregna-2,20(22)-dien-6-one **6**, m.p. 76-78°C (MeOH); IR (nujol) ν_{max} 1720 (-C=O), 1670, 1655cm^{-1}; ^{1}H-NMR δ 0.62 (s, 3H, 18-H_3), 0.75 (s, 3H, 19-H_3), 1.77 (s, 3H, 21-H_3), 4.64 and 4.8 (two bs, 2H, 22-H)), 5. 54-5.81 (m, 2H, 2-H and 3-H); m/z 312 (M^+), 297, 284, 269, 256, 243, 229, 213, 201, 185, 175, 159, 149, 133, 121, 107, 91(100%); Calc. for $C_{22}H_{32}O$ C, 84.61; H, 10.25. Found: C, 84.58; H, 10.58%; $[\alpha]_D^{25}$+11.2° (C 3, $CHCl_3$).

2α,3α-(20ζ)-20,22-Tetrahydroxypregna-6-one (7)

To a stirred solution of 2,20-diene **6** (0.312g, 1 mmol) in dry benzene (5 ml) was added OsO_4 (0.510g, 2 mmol) followed by 2 drops of pyridine and the reaction mixture was stirred at 25°C for 52h. It was then quenched with saturated aqueous sodium bisulphite solution. Benzene was removed under vacuo, the residue was dissolved in aqueous ethanol, sodium bisulphite was added and it was refluxed for 3h. Filtration followed by evaporation of ethanol gave a residue, which was extracted with ethyl acetate (3x50 ml). Usual work up followed by evaporation of solvent gave crude product, which after column chromatographic purification afforded tetrol **7** (0.273g, 72%), m.p. 254°C (ethyl acetate-methanol); IR ν_{max} 3480 (-OH) 1712 (-C=O)cm^{-1} ; ^{1}H-NMR δ 0.67 (s, 3H, 18-H_3), 0.77 (s, 3H, 19-H_3), 1.22 and 1.27 (s, 3H, 21-H_3), 3.1-3.9 (m, 4H, 2H, 3H, 22-H); m/z 380 (M^+), 362, 347, 329, 321, 285, 277, 267, 259, 245, 227, 210, 197, 187, 173, 159, 147, 133, 121, 105, 91(100%). Calc. for $C_{22}H_{36}O_5$ C, 69.4; H, 9.4; Found: C, 69.8; H, 9.8%.

3α,5-Cyclo-(20ζ)-20,22-dihydroxypregna-6-one (8)

To a stirred solution of keto olefin **5** (0.5g, 1.6 mmol) in dry benzene (7 ml) was added OsO_4 (0.41g, 1.6 mmol) followed by 2 drops of pyridine and the reaction mixture was stirred at 30°C for 22h. Benzene was removed under vacuo and the residue was dissolved in ethanol (12 ml). Sodium bisulphite was added and it was refluxed for 1h. Filtration followed by evaporation of ethanol gave a residue, which was extracted with ethyl acetate (3x50 ml). After usual work up ethyl acetate was evaporated to give the crude diol **8** (0.552g), which after column chromatographic purification over neutral alumina afforded pure **8** as gummy oil (0.4903g, 90%); IR ν_{max} 3450 (-OH), 1700 (-C=O)cm^{-1}; ^{1}H-NMR δ 0.92 and 1.02 (s, 3H, 18-H_3), 1.22 (s, 3H, 19-H_3), 1.68 and 1.70 (s, 3H, 21-H_3), 3.36-4.06 (m, 2H, 22-H).

3β-*tert*-Butyldimethylsiloxy-(20R)-20-hydroxydithianepregna-5-ene (10)

In a two necked flask equipped with a magnetic needle and a septum 1,3 dithiane (2.00g, 16.6mmol) was placed in dry THF (15ml). The solution was cooled to -30°C and n-BuLi in hexane (1.6M, 11ml) was added to it dropwise under nitrogen. The reaction mixture became pale yellow and it was stirred at this temperature for a period of 2h. To it compound **9**[13d] (3.75g, 8.7mmol) in anhydrous THF (20ml) was added dropwise at -30°C under nitrogen atmosphere. The reaction mixture was slowly brought to 0°C and was stirred at that temperature for an additional 24h. It was then quenched with cold saturated ammonium chloride solution and THF was removed under reduced pressure. The residue was extracted with ethyl acetate (4x50ml). Usual work up followed by evaporation of solvent under vacuo to afford 4.50g of crude product. Column chromatographic purification of the crude product over silica gel using ethyl acetate-pet ether (5:95) as eluent afforded pure **10** (3.88g, 81%) as a colourless solid. The solid was crystallised from CH_2Cl_2-hexane, m.p. 229-230°C; IR (nujol) ν_{max} 3458 (-OH) cm^{-1}; ^{1}H NMR (200 MHz) δ 0.07 (s, 6H, $SiMe_2$), 0.89 (s, 3H, 18-H_3), 0.91 (s, 9H, Si-*tert*-butyl), 1.02 (s, 3H, 19-H_3), 1.46 (s, 3H, 21-H_3), 2.77-3.06 (m, 4H, dithiane -CH_2), 3.40-3.60 (m, 1H, 3-H), 4.16 (s, 1H, dithiane -SCH), 5.33 (d, 1H, J=5Hz, 6-H); m/z 431 (M^+-dithiane), 373, 299, 159 (100%); Calc. for $C_{31}H_{54}O_2SiS_2$ C, 67.63; H, 9.68%; Found C, 67.91; H, 9.35%; $[\alpha]_D^{28}$-56.6° ($CHCl_3$, C=2.4).

(20R)-20,3β-Dihydoxy-20-dithianepregna-5-ene (11)

To a solution of compound **10** (1.4g, 2.5mmol) in dry THF, tetrabutylammonium fluoride (5ml, 1M solution in THF) was added and the reaction mixture was stirred at 30°C for 12h under nitrogen. Cold water was added to it and THF was removed from the reaction mixture under vacuum. The gummy mass was extracted with ethyl acetate (3x50ml). After usual work up ethyl acetate was evaporated under reduced pressure to give 1.346g of crude product which was chromatographed over silica gel using ethyl acetate-pet ether (1:4) to afford compound **11** (1.03g, 93%) as a pale yellow solid. This was crystallised from $CHCl_3$-hexane, m.p. 213-214°C (lit[15]. 213-214°C); IR (nujol) ν_{max} 3452 (-OH) cm^{-1}; ^{1}H NMR (200 MHz) δ 0.9 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 1.48 (s, 3H, 21-H_3), 2.81-3.04 (m, 4H. dithiane -SCH_2), 3.40-3.66 (m, 1H, 3-H), 4.16 (s, 1H, dithiane -SCH), 5.37 (d, 1H, J=5Hz, 6-H).

(20R)-20,3β-Dihydroxypregna-5-ene-22-aldehyde (12)

To a suspension of compound **11** (1.10g, 2.52mmol) in CH_3CN (80ml) and H_2O (20ml), HgO (0.720g, 3.32mmol) and $HgCl_2$ (1.21g, 4.45mmol) were added and the reaction mixture was refluxed for 7h with vigorous stirring. The solid mass was filtered, residue was thoroughly washed with ethyl acetate and the combined filtrate was dried over anhydrous sodium sulphate. Removal of solvent under reduced pressure gave a solid which was chromatographed over silica gel column using ethyl acetate-pet ether (1:3) to afford compound **12** (0.707g, 81%), crystallised from methanol, m.p.203-205°C (lit.[15] 205-207°C); IR (nujol) ν_{max} 3420 (-OH), 1720 (-CHO) cm^{-1}; ^1H NMR (200 MHz) δ 0.84 (s, 3H, 18-H_3), 1.04 (s, 3H, 19-H_3), 1.38 (s, 3H, 21-H_3), 3.43-3.64 (m, 1H, 3-H), 5.35 (d, 1H, J=5Hz, 6-H), 9.6 (s, 1H, -CHO).

3β-Tosyl-(20R)-20-Hydroxypregna-5-en-22-aldehyde (13)

To a solution of compound **12** (0.70g, 2mmol) in dry pyridine (5ml), *p*-TsCl (0.70g, 3.67mmol) was added at 0°C. The reaction reaction mixture was kept in dark for 12h at 30°C. It was then poured into 50ml of ice cold 10% $NaHCO_3$ solution and was kept for 2h with occassional stirring. The solid was extracted with diethyl ether (3x50ml). The ether layer was washed with water (2x50ml), $CuSO_4$ solution (3x50ml), water (2x50ml) and finally with brine (2x25ml). The organic layer was dried over anhydrous sodium sulphate and ether was removed under reduced pressure to afford the tosylate **13** (0.993g, 99%) as a pale yellow crystalline solid, m.p. 151-152°C; IR (nujol) ν_{max} 1720(-C=O), 1458 cm^{-1}; ^1H NMR (200 MHz) δ 0.8 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 1.37 (s, 3H, 21-H_3), 2.46 (s, 3H, tosyl CH_3), 3.23 (bs, 1H, -OH) 4.21-4.48 (m, 1H, 3-H), 5.34 (d, 1H, J=5Hz, 6-H), 7.35 and 7.80 (AB pattern, 4H, J=8Hz, Ar), 9.58 (s, 1H, -CHO).

3α, 5-Cyclo-6β-methoxy-(20R)-20-hydroxypregna-22-aldehyde (14)

To a solution of the tosylate **13** (0.97g, 1.95mmol) in dry methanol (50ml), fused KOAc (10g, 10mmol) was added and the reaction mixture was refluxed for a period of 4h. After the completion of the reaction, methanol was evaporated and the gummy mass was extracted with ethyl acetate (4x50ml). Usual work up followed by removal of ethyl acetate under vacuo gave the crude i-methyl ether **14** (1.2g). Column chromatographic purification over silica gel using ethyl acetate-pet ether as an eluent afforded the pure i-methyl ether **14** (0.523g, 75%) as a colourless foam which was crystallised from CH_2Cl_2-hexane, m.p. 144-145°C; IR (nujol) ν_{max} 1720 (-CHO) cm^{-1}; ^1H NMR (200 MHz) δ 0.45 (m, cyclopropyl-H), 0.70 (m, cyclopropyl-H), 0.86 (s, 3H, 18-H_3), 1.06 (s, 3H, 19-H_3), 1.3 (s, 3H, 21-H_3), 2.80 (bd, 1H, 6H), 3.35 (s, 3H, -OCH_3), 9.61 (s, 1H, -CHO); m/z 345 (M^+-CH_3); Calculated for $C_{23}H_{36}O_3$ C, 76.61; H, 10.06; Found C, 76.92; H, 10.17%;.

3α, 5-Cyclo-6β-methoxypregna-(20R)-20,22-diol (15)

To a suspension of lithium aluminium hydride (0.076g, 2mmol) in dry THF (5ml), compound **14** (0.360g, 1mmol) in dry THF (2ml) was added dropwise at 0°C under nitrogen. It was slowly brought to room temperature and was stirred for an additional 1h. The reaction mixture was again cooled to 0°C and excess lithium aluminium hydride was destroyed by careful addition of ethyl acetate. Cold saturated ammonium chloride solution was then added to it. The gelatinous mass was filtered through celite and the residue was thoroughly washed with ethyl acetate. The combined organic layer was then worked up as usual. Removal of solvent under vacuo furnished a thick oil which on column chromatographic purification on silica gel afforded pure **15** as a solid (0.340g, 94%), crystallised from ethyl acetate and hexane, m.p. 181-183°C; IR (nujol) ν_{max} 3400 (-OH) cm^{-1}; ^1H NMR (200 MHz) δ 0.46 (m, cyclopropyl-H), 0.67 (m, cyclopropyl-H), 0.95 (s, 3H, 18-H_3), 1.05 (s, 3H, 19-H_3), 1.34 (s, 3H, 21-H_3), 2.8 (m, 1H, 6-H), 3.35 (s, 3H, -OCH_3), 3.51 (d, 2H, J= Hz, 22-H_2); Calc. for $C_{23}H_{38}O_3$ C, 76.24; H, 10.49; Found C, 75.98; H, 10.55%.

3α, 5-Cyclo-6β-methoxypregna-(20R)-20,22-ethyl diether (16)

In a two necked flask, 50% mineral oil dispersion of NaH (0.024g, 0.50mmol) was placed under nitrogen. This was washed with dry hexane (3x2ml) and was then dried under vacuo. To it compound 15 (0.091g, 0.25mmol) in dry DMF (2ml) was added. The reaction mixture was stirred at 25°C for 1h and at 60°C for further 1h. It was

then cooled to 25°C, EtI (0.080g, 0.5mmol) was added dropwise and the reaction mixture was stirred at that temperature for an additional 5h for the reaction to complete. Water was added slowly and it was extracted with diethyl ether (3x30ml). After usual work up and removal of ether afforded a gum which was chromatographed over silica gel using ethyl acetate-pet ether (2:98) to furnish pure 16 (0.075g, 72%). This was crystallised from CH_2Cl_2-hexane to afford a low melting solid, m.p. 55-58°C; IR (nujol) vmax 2923, 2853, 1643, 1456 cm-1; 1H NMR (200 MHz) δ 0.45 (m, cyclopropyl-H), 0.67 (m, cyclopropyl-H), 0.90 (s, 3H, 18-H3), 1.05 (s, 3H, 19-H3), 1.24 (t, 6H, J=6Hz, 20 and 22 ethoxy CH3), 1.30 (s, 3H, 21-H3), 2.8 (m, 1H, 6-H), 3.20 (q, 2H, J=6Hz, 20 ethoxy -CH2), 3.35 (s, 3H, -OCH3), 3.53 (q, 2H, J=6Hz, 22 ethoxy -CH2); m/z 331 (M+-CH2OC2H5, -C2H4); Calc. for $C_{27}H_{46}O_3$ C, 77.45; H, 11.07; Found C, 77.33; H, 11.07%; $[\alpha]_D^{26}$-31.1° (C=2.7, CHCl3).

3β-Hydroxypregna-5-en-(20R)-20,22-ethyl diether (17)

To a solution of compound **16** (0.200g, 0.48mmol) in dioxane (6ml) and H_2O (1.5ml), PTSA (0.025g, 0.14mmol) was added and the reaction mixture was stirred at 60-65°C for 2h. Saturated aqueous $NaHCO_3$ solution was then added and the solvent was removed under reduced pressure. The solid mass was extracted with ethyl acetate (3x50ml) and the organic extract was worked up as usual. Removal of solvent afforded a yellowish gum which on chromatographic purification over silica gel using ethyl acetate-pet ether (1:9) afforded pure **17** (0.190g, 98%) as solid, m.p. 82°C (MeOH); IR (nujol) ν_{max} 3420 cm^{-1}; ^1H NMR (200MHz) δ 0.87 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 1.20 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.29 (s, 3H, 21-H_3), 3.20 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.50 (q, 2H, J=6Hz, 22 ethoxy -CH_2), 5.25 (d, 1H, J=5Hz, 6-H); Calc. for $C_{26}H_{44}O_3$ C, 77.22; H, 10.89; Found C, 77.03; H, 11.12%.

3β-Tosylpregna-5-en-(20R)-20,22-ethyl diether (18)

Tosylation of compound **17** (0.20g, 0.50mmol) afforded compound **18** (0.254g, 92%) as crystalline solid, m.p. 141-142°C; IR (nujol) ν_{max} 1610 cm^{-1}; $_1H$ NMR (200MHz) δ 0.86 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 1.24 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.29 (s, 3H, 21-H_3), 2.48 (s, 3H, tosyl -CH_3), 3.2 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.52 (q, 2H, J=6Hz, 22 ethoxy -CH_2), 4.25-4.55 (m, 1H, 3-H), 5.32 (d, 1H, J=5Hz, 6-H), 7.35 and 7.8 (AB pattern, 4H, J=8Hz, Ar).

3,α5-Cyclo-6β-hydroxypregna-(20R)-20,22-ethyl diether (19)

Solvolysis of the tosylate **18** (0.550g, 0.98mmol) in acetone (20ml), H_2O (5ml) and KOAc (0.600g, 6.12mmol) afforded a gum. This was chromatographed on silica gel using ethyl acetate-pet ether (2:8) to furnish pure **19** (0.330g, 83%) as foam which was crystallised from methanol to give crystalline solid, m.p. 167-169°C; IR (nujol)ν_{max} 3400 (-OH) cm^{-1}; ^1H NMR (200MHz) δ 0.36 (m, cyclopropyl-H), 0.55 (m, cyclopropyl-H), 0.95 (s, 3H, 18-H_3), 1.1 (s, 3H, 19-H_3), 1.23 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.30 (s, 3H, 21-H_3), 3.25 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.52 (q, 2H, J=6Hz, 22 ethoxy -CH_2)

3α,5-Cyclo-6-ketopregna-(20R)-20,22-ethyl diether (20)

Jones oxidation of the i-alcohol **19** (0.125g, 0.31mmol) in acetone (3ml) with chromic acid (8N, 0.2ml) in acetone (1ml) at 10-15°C for 15min followed by usual work up afforded the i-ketone **20** as gum. Chromatographic purification on silica gel using ethyl acetate-pet ether (5:95) gave pure **20** as solid (0.107g, 86%) which was crystallised from hexane, m.p. 105-108°C; IR (nujol) ν_{max} 1675 (-C=O) cm^{-1}; ^1H NMR (200MHz) δ 0.74 (m, 4-H_2), 0.91 (s, 3H, 18-H_3), 1.05 (s, 3H, 19-H_3), 1.25 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.3 (s, 3H, 19-H_3), 2.45 (d, 2H, 7-H_2), 3.22 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.53 (q, 2H, 22 ethoxy -CH_2); Calculated for $C_{26}H_{42}O_3$ C, 77.56; H, 10.51; Found C, 77.96; H, 10.73%; m/z 317 [M^+-($CH_2OC_2H_5$+ C_2H_4)]; $[\alpha]_D^{25}$= +39.06° (C=0.384, $CHCl_3$).

(20R)-20,22-Diethoxypregna-2-en-6-one (21)

To a solution of the ketone **20** (0.480g, 1.2mmol) in dry DMF (2ml), pyridinium *p*-toluenesulfonate (0.040g, 0.16mmol) and LiBr (0.04g, 0.46mmol) were added and the reaction mixture was heated under reflux for 4h. Water (10ml) was added and the gummy mass was extracted with diethyl ether (4x25ml). Usual work up and removal of ether under reduced pressure to afford a crude yellow gum which showed a complex mixture of products

on tlc. Column chromatographic purification on silica gel using ethyl acetate-pet ether (2:98) furnished pure **21** as foam (0.0816g, 17%). This was crystallised from CH_2Cl_2-hexane, m.p. 101-103°C; IR (nujol) ν_{max} 1705 cm^{-1}; ^1H NMR (200 MHz) δ 0.75 (s, 3H, 18-H_3), 0.79 (s, 3H, 19-H_3), 1.20 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.27 (s, 3H, 21-H_3), 3.24 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.52 (q, 2H, 6Hz, 22 ethoxy -CH_2), 5.61-5.85 (m, 2H, 2-H and 3-H); Calc. for $C_{26}H_{42}O_3$ C, 77.61; H, 10.45; Found C, 77.37; H, 10.60%.

2α,3α-Dihydroxy-6-ketopregna-(20R)-20,22-ethyl diether (22)

To a solution of 2-ene-6-keto compound **21** (0.076g, 0.19mmol) in acetone (3ml), was added OsO_4 (0.006g, 0.023mmol) in *tert*-butanol (0.15ml) followed by NMO (0.1g, 0.85mmol) in H_2O (0.15ml) and *tert*-butanol (0.2ml). The reaction mixture was stirred at 30°C under nitrogen for a period of 4h. It was quenched with aqueous 10% solution of $NaHSO_3$ and the mixture was filtered through celite. The residue was thoroughly washed with ethyl acetate and the combined organic washings were distilled off to obtain a coloured gum. This was extracted with ethyl acetate (4x25ml). Afetre usual work up solvent was removed under reduced pressure to give a colourless foam (0.077g, 94%) (single spot on tlc). The foamy mass was crystallised from ethyl acetate to furnish the 2α,3α-dihydroxy-6-ketopregna-(20R)-20,22-ethyl diether **22** as crystalline solid, m.p. 178°C; IR (nujol) ν_{max} 3300 (-OH), 2922, 1704 (-C=O) cm^{-1}; ^1H NMR δ 0.74 (s, 3H, 18-H_3), 0.79 (s, 3H, 19-H_3), 1.25 (t, 6H, J=6Hz, 20 and 22 ethoxy -CH_3), 1.35 (s, 3H, 21-H_3), 2.70 (bd, 1H, 5-H), 3.25 (q, 2H, J=6Hz, 20 ethoxy -CH_2), 3.53 (q, 2H, J=6Hz, 22- ethoxy -CH_2), 3.80 (m, 1H, 2-H), 4.07 (m, 1H, 3-H); m/z 391 (M^+- EtOH); Calc. for $C_{26}H_{44}O_5$ C, 71.55; H, 10.09; Found C, 71.23; H, 10.37%; $[\alpha]_D^{25}$= -8.46 (C=2.6, $CHCl_3$).

3β-Acetoxy-6-ketopregna-22-aldehyde (29a) and its C-20 epimer (29b)

To a solution of compound **20** (0.150g, 0.37mmol) in glacial acetic acid (1.3ml) was added H_2SO_4 (5N, 0.13ml) and the resulting greenish reaction mixture was stirred at 50-60°C for a period of 2h. The reaction was then quenched with saturated ice cold aqueous $NaHCO_3$ solution and the sticky mass was extracted with ethyl acetate (4x25ml). Usual work up of the organic extract and removal of solvent under reduced pressure furnished a deep yellow gummy mass which was chromatographed on silica gel to afford a mixture of **29a** and **29b** as colourless gum (0.144g, 68%), IR ν_{max} (nujol) 1723 (broad signal) cm^{-1}; ^1H NMR (200 MHz) δ 0.70 and 0.72 (s, 3H, 18-H_3), 0.81 and 0.85 ((s, 3H, 19-H_3), 1.05 and 1.07 (d, 2H, J=8Hz, 21-H_3), 2.05 (s, 3H, -$OCOCH_3$), 2.25-2.45 (dd, 2H, 7-H_2 of both the epimers), 4.56-4.80 (m, 1H, 3-H), 9..55 (d, 1H, J=8Hz, 22-H of one epimer) and 9.60 (d, 1H, J=8Hz, 22-H of the other epimer); m/z 388 (M^+), 373, 358, 344 (100%).

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