Synthesis of 3α,6β,17,20α-Tetrahydroxy-5β-pregnan-11-one 6-Hemisuccinate, a Hapten for Immunoassay of 3α,17,20α-Trihydroxy-5β-pregnan-11-one ('Pregnanetriolone')

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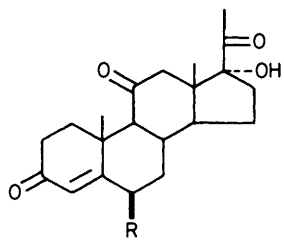
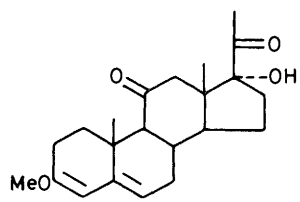
3α,6β,17,20α-Tetrahydroxy-5β-pregnan-11-one 6-hemisuccinate (**4**) has been synthesised by two distinct routes. In the first of these, introduction of a 6β-hydroxy substituent into 17α-hydroxypregn-4-ene-3,11,20-trione followed by catalytic hydrogenation of the Δ^4-olefinic bond gave 6β,17α-dihydroxy-5β-pregnane-3,11,20-trione. The 5β-configuration was confirmed by an *X*-ray crystallographic analysis of the derived 6-hemisuccinate. Subsequent selective reduction at C-3 and C-20 by sodium borohydride in the presence of cerous chloride gave the required compound (**4**), although in low overall yield. To avoid the problem of non-specificity in the reduction at C-20, alternative routes were explored from precursors already possessing the essential 17,20α-diol system, protected as the isopropylidene derivative or by acetylation as appropriate. Hydroboration–oxidation of 3,3-ethylenedioxy-17,20α-isopropylidenedioxypregn-5-en-11-one gave the 6β-hydroxy derivative of 5β-configuration, verified by dehydration ($POCl_3$–pyridine) to the 6-ene derivative. Further routine transformations including succinoylation of the 6β-hydroxy derivative, deprotection at C-3 and in the side chain, and reduction of the 3-oxo group, gave the required compound (**4**).

3α,17,20α-Trihydroxy-5β-pregnan-11-one ('pregnanetriolone') has not been detected in the urine of healthy subjects but has been observed in association with adrenal hyperplasia and the polycystic ovary syndrome.[1] Early identification of trace amounts of this abnormal metabolite would permit prompt diagnosis and treatment. The synthesis of the 6-hemisuccinate of the 6β-hydroxy derivative of this corticosteroid metabolite was undertaken in the hope of its providing a suitable hapten for urinary radioimmunoassay studies.

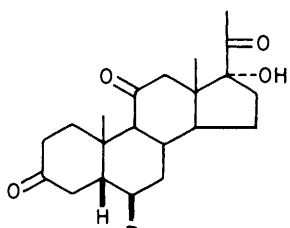


(1)

a; R = H
b; R = OH



(2)



(3)

a; R = OH
b; R = $OCOCH_2CH_2CO_2H$

Results and Discussion

17α-Hydroxypregn-4-ene-3,11,20-trione (**1a**) was envisaged as a suitable starting material for one route to the title compound. It was prepared from cortisone by removing the 21-hydroxy group by the method of Kočovský and Černý,[2] who studied the cortisol analogue, in 81% overall yield. Treatment with trimethyl orthoformate gave 17α-hydroxy-3-methoxypregna-3,5-diene-11,20-dione (**2**), which on irradiation in ethanol in the presence of air gave 6β,17α-dihydroxypregn-4-ene-3,11,20-trione (**1b**).

We had previously found that 6β-hydroxyprogesterone, with the same AB ring structure, undergoes stereoselective hydrogenation to furnish the corresponding 6β-hydroxy-5β-pregnan-3-one.[3] It was hoped that hydrogenation of (**1b**) would similarly give the 5β-steroid, although there seemed a possibility that the presence of the 11-oxo grouping in the molecule might influence the course of hydrogenation in favour of the 5α-isomer. Derks and Drayer[4] have indeed reported the synthesis of both 6β-hydroxy-5α- and 6β-hydroxy-5β-pregnan-11-ones after hydrogenation of 6β-hydroxypregn-4-ene-3,11-dione precursors. The hydrogenation of pregn-4-ene-3,11-diones in the absence of the 6β-hydroxy group is known, in some cases, to produce mainly the 5α-steroid.[5]

In the event, the hydrogenation on palladium–calcium carbonate afforded more than 54% of the required 5β-isomer. Assignment of the configuration at C-5 by the usual chiroptical method was considered unreliable in the presence of 11- and 20-oxo functions, which could mask the expected n→π* Cotton effect of the 3-oxo group. The ^1H n.m.r. spectrum was also not decisive in the absence of a pure sample of the 5α-isomer for reference. We therefore resorted to an *X*-ray crystallographic analysis, which was carried out following conversion of the reduced compound (**3a**) into its 6-hemisuccinate (**3b**), and confirmed the 5β-configuration beyond doubt (Figure).[6]

It is well established that reductions of 17α-hydroxy-20-oxo steroids with agents such as sodium borohydride normally give mainly the corresponding 17α-20β-diols and only poor yields of 17α,20α-diols. Before attempting such a reduction of the succinate (**3b**), it was important to establish the conditions which would maximise the yield of the 17α,20α-diol. To this end,

experiments with a variety of reducing systems were carried out employing 3β,17α-dihydroxypregn-5-en-20-one as a model compound. Paper chromatographic studies (Bush B4 solvent system [7]) proved particularly suitable in distinguishing between the epimeric products, pregn-5-ene-3β,17,20α- and pregn-5-ene-3β,17,20β-triols.

When zinc borohydride was employed as the reducing agent, only the 3β,17,20β-triol could be detected in the reaction mixture. With sodium borohydride as reducing agent, a number of additives were employed in the hope of promoting the formation of the 20α-hydroxy derivative, through complexation with the 17α-hydroxy and 20-oxo groups to rotate the pregnane side chain from its normal preferred configuration and expose the 'β'-face to attack. Additives included the lanthanide shift reagent tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium, simple metal halides including zinc chloride, and a number of chlorides of the lanthanide series including lanthanum and cerous chlorides. Of these, the last named gave the best results. In a series of experiments with the model steroid, yields in the range 36—45% of pregn-5-ene-3β,17,20α-triol were obtained, as estimated by g.l.c. studies. Accordingly, the reduction of the key intermediate (**3b**) was carried out with sodium borohydride in the presence of cerous chloride. The resulting mixture of 3,17,20-triols was subjected to partition chromatography on acid-washed Celite, the required 3α,17α,20α-isomer (**4**) being obtained in 23% yield from the trione (**3b**).

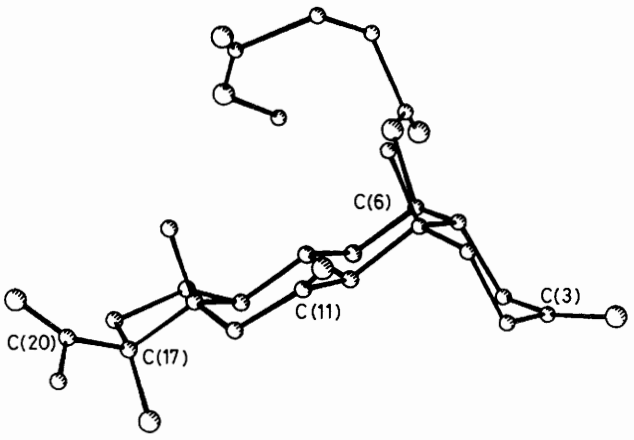
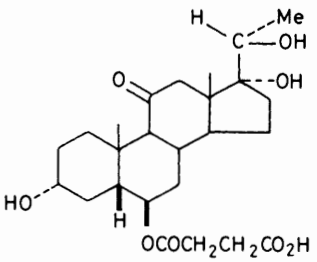
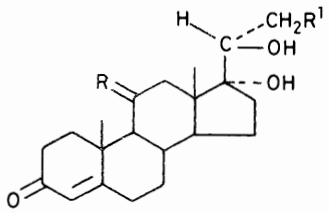


Figure.

One route to the required hapten having been established, we examined alternative procedures commencing with steroids known to contain the 17α,20α-dihydroxy side chain in order to avoid the final inefficient reductive step, and, if possible, to improve the overall yield. One such steroid employed for an initial study was 11β,17,20α,21-tetrahydroxypregn-4-en-3-one 21-acetate (**5a**). We first chose to protect the 17,20α-diol system as the isopropylidenedioxy derivative, hoping to achieve the reduction of the corresponding 21-methanesulphonate (**6a**) to

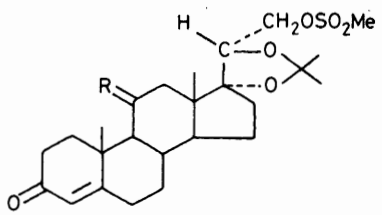


(4)



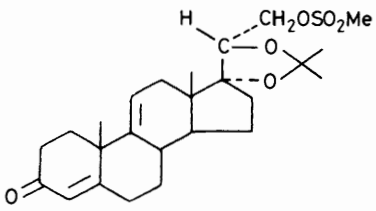
(5)

a; R = α-H, β-OH, R^1 = OAc
b; R = α-H, β-OH, R^1 = OH
c; R = O, R^1 = OSO_2Me



(6)

a; R = α-H, β-OH
b; R = O



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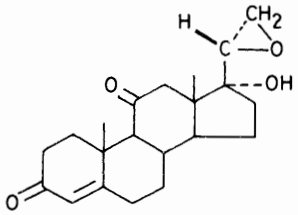
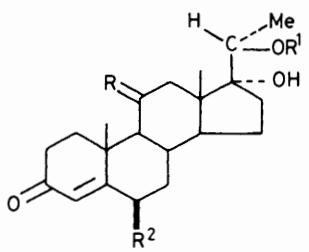
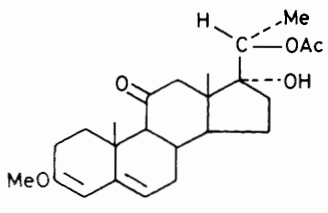


(8)



(9)

a; R = α-H, β-OH, R^1 = R^2 = H
b; R = O, R^1 = R^2 = H
c; R = α-H, β-OH, R^1 = Ac, R^2 = H
d; R = O, R^1 = Ac, R^2 = H
e; R = O, R^1 = Ac, R^2 = OH



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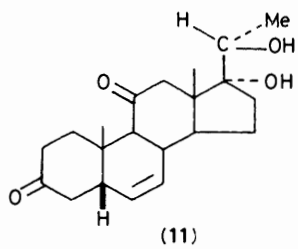


(11)

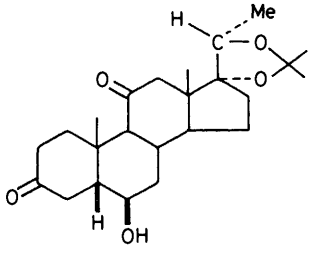
the 21-deoxy compound. However, we were unable to prepare the 21-methanesulphonate without concomitant dehydration occurring at C-11 to give the 9(11)-ene (**7**). In contrast, 17,20α-isopropylidenedioxy-21-methylsulphonyloxypregn-4-ene-3,11-dione (**6b**) was readily prepared, but the 21-substituent resisted all attempts at hydrogenolysis in the presence of the 17,20α-acetonide, probably for steric reasons.

As an alternative route from the protected compound (**6b**), hydrolysis of the acetonide with methanolic HCl gave 17,20α-dihydroxy-21-methylsulphonyloxypregn-4-ene-3,11-dione (**5c**) and this with methanolic potassium hydroxide [8] afforded 20α,21-epoxy-17α-hydroxypregn-4-ene-3,11-dione (**8**). Reduction with lithium aluminium hydride followed by selective re-oxidation at the allylic C-3 with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) gave 11β,17,20α-trihydroxypregn-4-en-3-one (**9a**). This and the related 17,20α-dihydroxypregn-4-ene-3,11-dione (**9b**), key intermediates in an alternative route to the title compound (**4**), were subsequently prepared routinely and more simply from cortisone *via* 17,21-isopropylidenedioxy-3-methoxypregna-3,5-diene-11,20-dione by the method of Nussbaum *et al.*,[9] thence by stereospecific reduction at C-20 according to Gardi *et al.*,[10] and deprotection to 11β,17,20α,21-tetrahydroxypregn-4-en-3-one. Removal of the 21-hydroxy group was then achieved by the method of Lewbart [11] in which selective conversion into the 21-toluene-*p*-sulphonate was followed by reduction with lithium aluminium hydride and selective reoxidation with DDQ, giving 11β,17,20α-trihydroxypregn-4-en-3-one (**9a**) in 21% overall yield from cortisone.

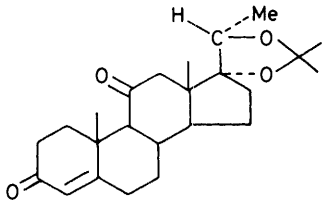
It was necessary next to introduce the 6β-hydroxy substituent *via* a 3,5-dien-3-yl ether derivative. An attempt to obtain 17,20α-isopropylidenedioxy-3-methoxypregna-3,5-dien-11β-ol from compound (**9a**) in one stage, by the method of Nussbaum *et al.*,[9] was unsuccessful, as were attempts to prepare the corresponding 11-oxo analogue. Two-stage preparations *via* the known 17,20α-isopropylidenedioxy-4-en-3-ones were likewise unsuccessful, so we were led to consider alternative means for protecting the dihydroxy side chain. Mild acetylation at C-20 in both the 11β-hydroxy (**9a**) and 11-oxo compounds (**9b**) gave the respective 20α-acetoxy derivatives (**9c**) and (**9d**). The 11β-hydroxy compound (**9c**) was converted smoothly into the 11-ketone (**9d**) under the mild oxidation conditions afforded by chromium trioxide in pyridine, or by pyridinium chlorochromate.[12]

Initial attempts to convert the 11-oxo-20α-acetoxy compound into the corresponding 3-enol methyl ether (**10**) were complicated by the formation of by-products, but the preparation was subsequently accomplished when the reaction was carried out in the dark and under nitrogen. It is well known (*cf.* especially Gardi and Lusignani [13]) that many 3,5-dien-3-ol ethers undergo ready auto-oxidation; the present compound (**10**) was clearly exceptionally prone to this reaction. Irradiation of an ethanolic solution of the enol ether gave an acceptable yield of the 6β-hydroxy-4-en-3-one (**9e**). Following the preparation of this compound, retrospective analysis of the products resulting from earlier attempts to prepare the 3,5-dienol ether revealed low yields of this and the 6β-hydroxy-4-en-3-one, together with other unidentified products.

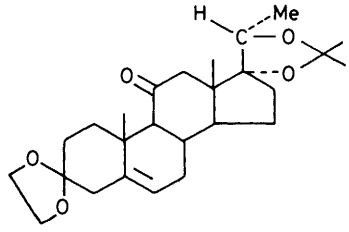
As in the earlier series (see p. 1205) hydrogenation of the 6β-



(12)



(13)



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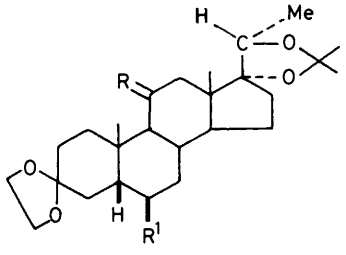
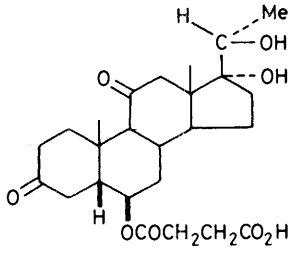


(15)

a; R = α-H, β-OH, R^1 = OH
b; R = O, R^1 = OAc
c; R = O, R^1 = OH
d; R = α-H, β-OH, R^1 = OAc



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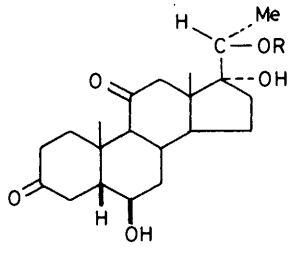
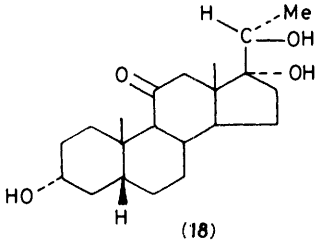


(17)

a; R = Ac
b; R = H



(18)

hydroxy-4-en-3-one (**9e**) was attempted in pyridine employing palladium on calcium carbonate as catalyst and afforded more than 75% * of a single isomer (**17a**) at C-5 which was examined further as follows. Hydrolysis of the 20α-acetoxy grouping followed by formation of the 17,20α-acetonide, treatment in pyridine with phosphorus oxychloride, and hydrolysis of the acetonide, gave a good yield of a 6-ene. This could only have arisen from a 6β-hydroxy-5β-pregnane,[14] and established the structure of the dehydration product as 17,20α-dihydroxy-5β-pregn-6-ene-3,11-dione (**11**). Further confirmation was obtained by hydrogenation of the 6-ene followed by reduction of the 3-oxo group with sodium borohydride to give 3α,17,20α-trihydroxy-5β-pregnan-11-one (**18**), identical with an authentic sample of 'pregnanetriolone'. 6β-Hydroxy-17,20α-isopropylidenedioxy-5β-pregnane-3,11-dione (**12**) is thus a further intermediate in the synthesis of the required 3α,6β,17,20α-tetrahydroxy-5β-pregnan-11-one 6-hemisuccinate (**4**), being converted into the latter by succinoylation at C-6 followed by acid hydrolysis of the protecting acetal group and mild reduction to obtain the equatorial 3α-hydroxy derivative.

Another route to the title compound, aimed at introducing the 6β-hydroxy-5β-H features by a stereochemically unambiguous route, involved the hydroboration–oxidation of 3,3-ethylenedioxy-17,20α-isopropylidenedioxypregn-5-en-11-one (**14**) prepared in the usual way[15] from 17,20α-isopropylidenedioxypregn-4-ene-3,11-dione (**13**). Hydroboration of 3,3-ethylenedioxy-5-enes is known to favour β-face addition for steric reasons.[14] Reaction with diborane followed by alkaline hydrogen peroxide gave 3,3-ethylenedioxy-17,20α-isopropylidenedioxy-5β-pregnane-6β,11β-diol (**15a**). Mild acetylation (at C-6 only), followed by pyridinium chlorochromate oxidation then gave 6β-acetoxy-3,3-ethylenedioxy-17,20α-isopropylidenedioxy-5β-pregnan-11-one (**15b**). Alkaline hydrolysis furnished the 6β-hydroxy compound (**15c**) which with succinic anhydride formed the 6β-hemisuccinate. Acidic hydrolysis of the protecting acetal groups then gave 6β,17,20α-trihydroxy-5β-pregnane-3,11-dione 6-hemisuccinate (**16**). Reduction of the 3-oxo group as before with sodium borohydride, followed by purification by partition chromatography on Celite, gave 3α,6β,17,20α-tetrahydroxy-5β-pregnan-11-one 6-hemisuccinate (**4**), identical in all respects with that obtained by the previous route (see p. 1206).

For further confirmation of the 6β-hydroxy-5β-configuration, 3,3-ethylenedioxy-6β-hydroxy-17,20α-isopropylidenedioxy-5β-pregnan-11-one (**15c**) was treated with phosphorus oxychloride in pyridine and the product was hydrolysed with aqueous acetic acid to give 17,20α-dihydroxy-5β-pregn-6-ene-3,11-dione (**11**), identical with that prepared by the route described above.

The alternative path to product (**4**) offered no advantage over our original route from the hydroxytrione (**1**), and involved more steps. The hemisuccinate (**4**) is being used to raise an antibody to pregnanetriolone.

A point of interest arising from the ^{1}H n.m.r. spectra of the 17α-hydroxy-11-ones carried out in pyridine was the appearance, in each case, of a broad one-proton doublet (*J ca.* 12 Hz) in the region 3.20—3.40 p.p.m. which we assigned to the 12β-proton. N.O.e. experiments on similarly substituted compounds,[16] involving irradiation of the adjacent 18-Me, confirmed the assignment. We attribute the exceptional chemical shift to the association of the 17-hydroxy group with pyridine[17] as we have not observed it in other solvents.

The ^{13}C n.m.r. data are entirely consistent with the 5β-configuration (see Experimental section).

* Subsequent fractionation of the residual *ca.* 25% and examination by ^{1}H and ^{13}C n.m.r. spectroscopy failed to provide evidence for the 5α-isomer.

Experimental

M.p.s were measured on a Reichert microscope melting point apparatus and are corrected. I.r. spectra were recorded on a Perkin-Elmer 237 Grating Infrared spectrophotometer or on a Pye Unicam SP 3-200 Infrared spectrophotometer. N.m.r. spectra were obtained with a Jeol FX100 spectrophotometer at 100 MHz. The 400 MHz spectrum was obtained on the University of London Intercollegiate Research Service Bruker WH-400 spectrometer at Queen Mary College. Chemical shifts are reported in p.p.m. (δ) relative to internal Me_4Si. Microanalyses were carried out by the Microanalytical Service, Chemistry Department, University College, London WC1H 0AJ. The mass spectrum was obtained on the University of London Intercollegiate Research Service VG Analytical Instrument ZAB-IF at the School of Pharmacy.

17α-*Hydroxypregn*-4-*ene*-3,11,20-*trione* (**1a**).—17α-Hydroxy-21-methylsulphonyloxypregn-4-ene-3,11,20-trione[18] (10.22 g) in 1,2-dimethoxyethane (171 ml) with sodium iodide (10.22 g), zinc powder (10.22 g), and water (10.22 ml) were stirred and heated under reflux for 5 h. Isolation of the product with ethyl acetate and purification from methanol gave 17α-hydroxypregn-4-ene-3,11,20-trione as prisms (7.14 g, 89%), m.p. 224—229 °C (decomp.) (lit.,[19] m.p. 236—239 °C); $\nu_{max.}$(KBr) 3 560 (OH), 1 705, 1 700 (11- and 20-CO), and 1 660 and 1 620 cm^{-1} (4-en-3-one); δ($CDCl_3$) 0.72 (s, 18-H_3), 1.40 (s, 19-H_3), 2.25 (s, 21-H_3), 3.04 (s, 17-OH), and 5.72 (s, 4-H).

17α-*Hydroxy*-3-*methoxypregna*-3,5-*diene*-11,20-*dione* (**2**).—17α-Hydroxypregn-4-ene-3,11,20-trione (7.0 g) in dry dioxane (175 ml) with trimethyl orthoformate (8.75 ml) and toluene-*p*-sulphonic acid (350 mg) was stored at room temperature for 1.25 h and then added to a solution of sodium hydrogen carbonate (10.5 g) in water (1.25 l). The precipitated solid was purified from aqueous methanol to give the *enol ether* (**2**) as rods (5.57 g, 76.5%), m.p. 146—152 °C, $\nu_{max.}$(KBr) 3 510 (OH), 1 700, 1 692 (CO), and 1 655 and 1 630 cm^{-1} (3,5-diene); δ($CDCl_3$ + trace C_5D_5N) 0.67 (s, 18-H_3), 1.15 (s, 19-H_3), 2.24 (s, 21-H_3), 3.55 (s, 3-OMe), 5.09 (s, 4-H), and 5.19 (m, 6-H).

6β,17α-*Dihydroxypregn*-4-*ene*-3,11,20-*trione* (**1b**).—17α-Hydroxy-3-methoxypregna-3,5-diene-11,20-dione (5.57 g) in ethanol (557 ml, redistilled over KOH) was magnetically stirred and irradiated during 5 h with a Philips Ultraphil Health Lamp (type KL 2866). Rotary evaporation gave a crystalline solid which was purified from acetone to give the 6β-*hydroxy derivative* (**1b**) (2.41 g, 45.6%), m.p. 229—237 °C (decomp.); $\nu_{max.}$(KBr) 3 500sh and 3 430 (OH), 1 700 (CO), and 1 662 and 1 618 cm^{-1} (4-en-3-one); δ($CDCl_3$ + trace C_5D_5N) 0.72 (s, 18-H_3), 1.62 (s, 19-H_3), 2.25 (s, 21-H_3), 4.38 (t, *w ca.* 7 Hz, 6α-H), 4.90 (br s, 6-OH, removed by D_2O), and 5.79 (s, 4-H) (Found: C, 69.5; H, 7.8. $C_{21}H_{28}O_5$ requires C, 70.0; H, 7.8%).

6β,17α-*Dihydroxy*-5β-*pregnane*-3,11,20-*trione* (**3a**).—6β,17α-Dihydroxypregn-4-ene-3,11,20-trione (2.674 g) in freshly redistilled pyridine (45 ml) was hydrogenated in the presence of palladium (5% on $CaCO_3$; 2.674 g). Filtration of the catalyst followed by isolation with ethyl acetate gave crude 6β,17α-*dihydroxy*-5β-*pregnane*-3,11,20-*trione* (**3a**) (2.32 g), m.p. 230—250 °C. Purification from acetone–hexane gave square prisms (1.43 g, 54%), m.p. 256—260 °C; $\nu_{max.}$(KBr) 3 490 and 3 450sh (OH), 1 715sh, and 1 710 and 1 685 cm^{-1} (CO); δ($CDCl_3$ + trace C_5D_5N) 0.68 (s, 18-H_3), 1.29 (s, 19-H_3), 2.24 (s, 21-H_3), 3.76 (s, 6α-H), and 5.84 (br, 6β-OH, removed by D_2O); Δε +2.67 (305 nm) (*c*, 0.40 in methanol) (Found: C, 69.3; H, 8.3. $C_{21}H_{30}O_5$ requires C, 69.6; H, 8.3%).

6β,17α-*Dihydroxy*-5β-*pregnane*-3,11,20-*trione* 6-*Hemisuccinate* (**3b**).—6β,17α-Dihydroxy-5β-pregnane-3,11,20-trione (200

mg) in dry pyridine (2.0 ml) with succinic anhydride (400 mg; twice recrystallised) was heated on the steam bath for 5 h then the pyridine was removed under reduced pressure. Purification of the semi-crystalline mass from aqueous methanol gave the 6-*hemisuccinate* (**3b**) as cubes (87.6 mg, 34.3%), m.p. 145—147 °C; $\nu_{max.}$(KBr) 3 485 (OH), 1 725sh and 1 705 (succinoyl and CO), and 1 175 cm^{-1} (succinoyl); δ($CDCl_3$) 0.75 (s, 18-H_3), 1.33 (s, 19-H_3), 2.28 (s, 21-H_3), 2.66 (s, $CH_2CH_2CO_2H$), and 4.73 (s, *w* 7 Hz, 6α-H). The structure of this compound was confirmed by *X*-ray crystallographic analysis (see Figure).

3α,6β,17,20α-*Tetrahydroxy*-5β-*pregnan*-11-*one* 6-*Hemisuccinate* (**4**).—To a solution of cerous chloride heptahydrate (450 mg) in methanol (3.0 ml) was added 6β,17α-dihydroxy-5β-pregnane-3,11,20-trione 6-hemisuccinate (128 mg). Sodium borohydride (69 mg) was then added in portions during 10 min and the progress of the reaction was followed by t.l.c. After 5 h at room temperature further cerous chloride heptahydrate (450 mg) in methanol (3.0 ml) was added followed by sodium borohydride (33 mg) and the mixture was left overnight at room temperature. Acetic acid was then added to bring the mixture to pH 4.0. Dilution with water and isolation with ether gave a colourless glass (136 mg). Partition chromatography on acid-washed Celite 535 (10.0 g) was carried out employing the system t-butyl alcohol (10%), toluene (40%), acetic acid (15%), and water (35%). Fractions of 10 ml were collected, nos. (18—23) being combined to give the title compound (30 mg, 23%). This could not be crystallised but gave a solid, m.p. 125—129 °C, upon trituration with n-hexane; $\nu_{max.}$(KBr) 3 440 (OH), 1 720sh ($OCOCH_2CH_2CO_2H$), 1 700 (CO), and 1 170 cm^{-1} ($OCOCH_2CH_2CO_2H$); δ(C_5D_5N), 0.81 (s, 18-H_3), 1.41 (d, *J ca.* 6.5 Hz, 21-H_3), 1.64 (s, 19-H_3), 2.92 (s, $CH_2CH_2CO_2H$), 3.30 (d, *J ca.* 12 Hz, 12β-H), 3.80 (m, 3β-H) overlapping 4.01 (q, *J ca.* 6 Hz, 20β-H), and 5.02 (s, *w* 8 Hz, 6α-H). The overlapping 3β-H and 20β-H signals were fully resolved in a 400 MHz spectrum obtained for the same solution using a Bruker WH-400 spectrometer.* The 400 MHz spectrum also showed the hemisuccinate methylene proton signals as distinct triplets at δ 2.91 and 2.96.

17,20α-*Isopropylidenedioxy*-21-*methylsulphonyloxypregna*-4,9(11)-*dien*-3-*one* (**7**).—To 11β,21-dihydroxy-17,20α-isopropylidenedioxypregn-4-en-3-one (228.2 mg) in dry pyridine (1.0 ml) stirred and cooled in ice, was added methanesulphonyl chloride (0.5 ml). After 2 days at room temperature the mixture was diluted with iced water and the solid purified from methanol to give 17,20α-*isopropylidenedioxy*-21-*methylsulphonyloxypregna*-4,9(11)-*dien*-3-*one* (**7**) as rectangular prisms (107.1 mg, 40.9%), m.p. 179—182 °C; $\nu_{max.}$(KBr) 1 660, 1 610, 1 240, 1 219, 1 180, 1 149, 1 003, and 960 cm^{-1}; δ($CDCl_3$ + trace C_5D_5N) 0.83 (s, 18-H_3), 1.35 (s, 19-H_3), 1.49 (acetonide-H_6), 3.13 (s, mesylate-H_3), 4.47 (m, 20 and 21-H_3), 5.52 (d, *J* 6 Hz, 11-H), and 5.76 (s, 4-H) (Found: C, 64.8; H, 7.8; S, 7.2. $C_{25}H_{36}O_6S$ requires C, 64.6; H, 7.8; S, 6.9%).

17,20α-*Isopropylidenedioxy*-21-*methylsulphonyloxypregn*-4-*ene*-3,11-*dione* (**6b**).—21-Hydroxy-17,20α-isopropylidenedioxypregn-4-ene-3,11-dione (1.11 g) in dry pyridine (5.5 ml) was stirred at 0 °C and treated with methanesulphonyl chloride (2.75 ml). After 2 days at room temperature dilution with iced water and purification from methanol gave the 21-*methanesulphonate* (**6b**) (0.82 g, 62%), m.p. 172—174 °C (decomp.); $\nu_{max.}$(KBr) 1 700 (CO), 1 665, 1 615, 1 235, 1 175, and 955 cm^{-1}; δ($CDCl_3$ + trace C_5D_5N) 0.82 (s, 18-H_3), 1.40 (s, 19-H_3), 1.45 (s, acetonide-H_6), 3.08 (s, mesylate-H_3), 4.40 (m, 20-H + 21-H_2), and 5.70 (s, 4-H) (Found: C, 62.4; H, 7.5; S, 6.8. $C_{25}H_{36}O_7S$ requires C, 62.5; H, 7.55; S, 6.7%).

17,20α-*Dihydroxy*-21-*methylsulphonyloxypregn*-4-*en*-3,11-*dione* (**5c**).—17,20α-Isopropylidenedioxy-21-methylsulphonyloxypregn-4-ene-3,11-dione (299 mg) in methanol (56 ml) with concentrated HCl (28 ml) was heated at 100 °C for 10 min. Dilution with water, isolation *via* dichloromethane, and purification from methanol gave the 21-*methanesulphonate* (**5c**) as blades (122 mg, 44.5%), m.p. 173—174 °C (decomp.); $\nu_{max.}$(KBr) 3 510 (OH), 1 700 (CO), 1 658 and 1 611 (4-en-3-one), and 1 170 cm^{-1} (methanesulphonate); δ(C_5D_5N) 0.94 (s, 18-H_3), 1.42 (s, 19-H_3), 3.20 (d, *J ca.* 12 Hz, 12β-H), 3.28 (s, mesylate-H_3), 4.36 (m, *w* 16 Hz, 20β-H), 4.86 (m, *w* 12 Hz, 21-H_2), 5.86 (s, 4-H), and 6.39 (s, 17-OH) (Found: C, 60.35; H, 7.5; S, 7.0. $C_{22}H_{32}O_7S$ requires C, 60.0; H, 7.3; S, 7.3%).

20α,21-*Epoxy*-17α-*hydroxypregn*-4-*ene*-3,11-*dione* (**8**).—17,20α-Dihydroxy-21-methylsulphonyloxypregn-4-ene-3,11-dione (**5c**) (143 mg) in methanol (11.5 ml) with aqueous 1M-potassium hydroxide (1.0 ml) was stored at room temperature for 3 h. Water (100 ml) was added and the product was isolated with dichloromethane and purified from acetone–hexane to give the 20α,21-*epoxide* (**8**) as prisms (44 mg, 39.4%), m.p. 182—184 °C; $\nu_{max.}$(KBr) 1 690 (CO), 1 650 and 1 615 cm^{-1} (4-en-3-one); δ($CDCl_3$) 0.83 (s, 18-H_3), 1.41 (s, 19-H_3), 2.98 (dd, $J_{20.21a}$ 6 Hz, $J_{20.21b}$ 4 Hz, 20β-H), 3.72 (m, 21-H_2 + 17-OH, removed by D_2O), and 5.73 (s, 4-H) (Found: C, 73.0; H, 8.1. $C_{21}H_{28}O_4$ requires C, 73.2; H, 8.2%).

11β,17,20α-*Trihydroxypregn*-4-*ene*-3-*one* (**9a**).—20α,21-Epoxy-17α-hydroxypregn-4-ene-3,11-dione (**8**) (96 mg) in dry benzene (10 ml) was added to $LiAlH_4$ (45 mg) in dry ether (15 ml) and the mixture was heated under reflux for 30 min. Filtration through a pad of Celite followed by extensive washing with ether and dichloromethane, and evaporation of these solvents, gave a partially crystalline gum (89 mg). This in dioxane (1 ml) was treated with DDQ (90 mg). After 40 h at room temperature the mixture was diluted with dichloromethane and washed with aqueous sodium hydroxide (10%) then with brine and dried ($NaSO_4$). Evaporation of the solvents gave the crude 11β,17,20α-triol (**9a**) (60 mg) which was purified in ethyl acetate through a short column of silica gel followed by crystallisation from ethyl acetate (24 mg, 24.7%), m.p. 188—191 °C; raised to 194—197 °C by a further recrystallisation (lit.,[11] m.p. 202—204 °C); δ($CDCl_3$) 1.01 (s, 18-H_3), 1.21 (d, *J* 6.5 Hz, 21-H_3), 1.45 (s, 19-H_3), 1.60 (s, 17- and 20α-OH), 1.92 (s, 11β-OH), 3.87 (m, *w ca.* 18 Hz, 20-H), 4.40 (m, *w* 12 Hz, 11α-H), and 5.67 (s, 4-H).

11β,17,20α-*Trihydroxypregn*-4-*en*-3-*one* 20-*Monoacetate* (**9c**).—11β,17,20α-Trihydroxypregn-4-en-3-one (540 mg) in pyridine (10.8 ml) and acetic anhydride (8.0 ml) were allowed to react for 18.5 h, then poured into iced water. The 20-*acetate* (**9c**) gave rods (492 mg, 81.3%), m.p. 217—219 °C from acetone–hexane; $\nu_{max.}$(KBr) 3 500 and 3 460 (OH), 1 708 (OAc), 1 660 and 1 612 (4-en-3-one), and 1 275 cm^{-1} (OAc); δ(C_5D_5N) 1.35 (s, 18-H_3), 1.47 (d, *J* 6 Hz, 21-H_3), 1.61 (s, 19-H_3), 1.98 (s, OAc), 4.58 (m, 11α-H), 5.50 (m, 11-OH + 20β-H), 5.66 (s, 17α-OH), and 5.84 (s, 4-H) (Found: C, 70.3; H, 8.8. $C_{23}H_{34}O_5$ requires C, 70.7; H, 8.8%).

17,20α-*Dihydroxypregn*-4-*ene*-3,11-*dione* 20-*Monoacetate* (**9d**).—11β,17,20α-Trihydroxypregn-4-en-3-one 20-monoacetate (240 mg) in dichloromethane (6 ml) was added with stirring to pyridinium chlorochromate (150 mg) in dichloromethane (1 ml). After 2 h the mixture was diluted with ether and filtered through a pad of Florisil which was washed with more ether and dichloromethane. The filtrate and washings were

* We are grateful to Dr. G. E. Hawkes, Queen Mary College, University of London, for this spectrum.

evaporated and the light brown gum in ethyl acetate was filtered through a short column of Florisil to give the 20-*acetate* (**9d**) (200 mg, 83.8%), m.p. 184—189 °C (from acetone–hexane). The same product was obtained by direct acetylation of 17,20α-dihydroxypregn-4-ene-3,11-dione; $\nu_{max.}$(KBr) 3 550 (OH), 1 730 (OAc), 1 690 (11-CO), 1 670 and 1 615 (4-en-3-one), and 1 255 cm^{-1} (OAc); δ(C_5D_5N) 0.80 (s, 18-H_3), 1.39 (d, *J* 8 Hz, 21-H_3), 1.43 (s, 19-H_3), 1.95 (s, OAc), 3.22 (d, *J ca.* 12 Hz, 12β-H), 5.35 (q, *J* 7 Hz, 20β-H), 5.85 (s, 4-H), and 6.33 (s, 17-OH) (Found: C, 70.8; H, 8.3. $C_{23}H_{32}O_5$ requires C, 71.1; H, 8.3%).

20α-*Acetoxy*-17-*hydroxy*-3-*methoxypregna*-3,5-*dien*-11-*one* (**10**).—17,20α-Dihydroxypregn-4-ene-3,11-dione 20-monoacetate (100 mg) in dioxane (2.0 ml) with trimethyl orthoformate (0.4 ml) and toluene-*p*-sulphonic acid (5.0 mg) was stored under nitrogen in the dark for 2 h. Pyridine (0.05 ml) was then added and the mixture was poured into iced water. Purification of the solid in the dark from methanol containing a trace of pyridine gave the dienol ether (**10**) as prisms (80 mg, 68.9%), m.p. 121—126 °C; $\nu_{max.}$(KBr) 3 460 (OH), 1 730 (OAc), 1 700 (CO), 1 652 and 1 630 (3,5-diene), and 1 242 cm^{-1} (OAc); δ(C_5D_5N) 0.83 (s, 18-H_3), 1.38 (s, 19-H_3), 1.41 (d, *J* 6 Hz, 21-H_3), 1.95 (s, OAc), 3.26 (d, *J* 12 Hz, 12β-H), 3.52 (s, OMe), 5.27 and 5.39 (m, 4-H and 6-H), and 6.22 (s, 17-OH). An earlier similar preparation carried out in daylight, with exposure to air, gave only a very poor yield (*ca.* 10%) of the required enol ether and a similar yield of a compound with a higher m.p. (235—240 °C). This subsequently proved to be the 6β-hydroxy-4-en-3-one (**9e**). The enol methyl ether is thus very susceptible to autoxidation. The thoroughly dried enol methyl ether was stable in the dark for more than 11 months.

A satisfactory elemental analysis could not be obtained for the compound presumably owing to the rapid autoxidation.

20α-*Acetoxy*-6β,17-*dihydroxypregn*-4-*ene*-3,11-*dione* (**9e**).—The dienol ether (**10**) (415 mg) in ethanol (42.0 ml; previously redistilled over KOH) was stirred and irradiated with a Philips Ultraphil Health Lamp (type KL 2866) during 2.5 h. Rotary evaporation followed by purification of the residue from acetone gave the 6β-*hydroxy derivative* (**9e**) as plates (166 mg, 39.8%), m.p. 271—276 °C; $\nu_{max.}$(KBr) 3 440 and 3 400 (OH), 1 730 (OAc), 1 710 (CO), 1 665 and 1 620 (4-en-3-one), and 1 252 cm^{-1} (OAc); δ(C_5D_5N) 0.84 (s, 18-H_3), 1.38 (d, *J* 6 Hz, 21-H_3), 1.93 (s, 6 H, 19-H_3 and OAc), 3.26 (d, *J* 12 Hz, 12β-H), 4.53 (m, 6α-H), 5.33 (q, *J* 7 Hz, 20β-H), 6.03 (s, 4-H), 6.28 (s, 17-OH), and 6.98 (m, 6β-OH) (Found: C, 68.15; H, 7.8. $C_{23}H_{32}O_6$ requires C, 68.3; H, 8.0%).

20α-*Acetoxy*-6β,17-*dihydroxy*-5β-*pregnane*-3,11-*dione* (**17a**).—20α-Acetoxy-6β,17-dihydroxypregn-4-ene-3,11-dione (**9e**) (180 mg) in freshly redistilled pyridine (4.0 ml) was hydrogenated over palladium (5%) on $CaCO_3$ (180 mg). After separation from the catalyst the product was isolated with ethyl acetate to give the 5β-*dihydro derivative* (**17a**) as needles (135 mg, 75%), m.p. 277—283 °C; $\nu_{max.}$(KBr) 3 522 and 3 430 (OH), 1 728 (OAc), 1 705 (CO), and 1 254 cm^{-1} (OAc); δ(C_5H_5N) 0.84 (s, 18-H_3), 1.39 (d, *J* 6 Hz, 21-H_3), 1.86 (s, 19-H_3), 1.94 (s, OAc), 3.38 (d, *J* 12 Hz, 12β-H), 3.92 (m, *w* 8 Hz, 6α-H), 5.34 (q, *J* 6 Hz, 20β-H), and 6.26 (overlapping m, s, 6β + 17-OH) (Found: C, 67.8; H, 8.2. $C_{23}H_{34}O_6$ requires C, 67.95; H, 8.4%).

17,20α-*Dihydroxy*-5β-*pregn*-6-*ene*-3,11-*dione* (**11**).—20α-Acetoxy-6β,17-dihydroxy-5β-pregnane-3,11-dione (**17a**) (30 mg) in ethanol (4.5 ml) with potassium hydrogen carbonate (30 mg) in water (0.15 ml) was heated under reflux for 1 h, then the product was isolated *via* ethyl acetate. The resulting crude triol (**17b**) crystallised upon trituration with acetone (28.5 mg), m.p. 186—191 °C; $\nu_{max.}$(KBr) 3 500sh, 3 440, and 3 300 (OH), and 1 700 cm^{-1} (CO); δ(C_5D_5N) 0.87 (s, 18-H_3), 1.44 (d, *J ca.* 6.5 Hz, 21-H_3), 1.89 (s, 19-H_3), 3.41 (d, *J ca.* 12 Hz, 12β-H), 3.92 (m, *w* 7 Hz, 6α-H), 4.07 (q, *w ca.* 7 Hz, 20β-H), 5.29 (s, 17-OH), 5.90 (m, 6β-OH), and 6.28 (d, *J ca.* 4 Hz, 20α-OH). The crude triol was converted into the 17,20α-acetonide (**12**) in acetone (6.0 ml) with concentrated HCl (0.03 ml) during 18 h at room temperature [$\nu_{max.}$(KBr) 3 450 (OH), 1 702 (CO), 1 240, 1 214, and 1 010 cm^{-1} (acetonide)] and the product (28 mg) was dehydrated in pyridine (0.3 ml) with phosphorus oxychloride (0.045 ml) to give the crude 6-ene (20.1 mg); $\nu_{max.}$(KBr) 3 020 (6-ene), 1 710 (CO), 1 240, 1 216, and 1 010 cm^{-1} (acetonide). This, in 60% acetic acid (3.0 ml) to remove the acetonide group, was stored at room temperature for 91 h, and the solvents were then evaporated under reduced pressure. The product crystallised upon trituration with methanol. Purification from ethanol gave the 6-ene (**11**) as blades (3.3 mg), m.p. 218—221 °C. The filtrate was evaporated to dryness and the residue was purified by partition chromatography on a column of acid-washed Celite 535 (4.0 g) employing the Bush B2[7] solvent system. Fractions of 2 ml were collected, fractions 10—15 giving a further 6.3 mg of the pure 6-ene. [The total yield (9.6 mg) respresents 37.6% from 20α-acetoxy-6β,17-dihydroxy-5β-pregnane-3,11-dione (**17**).] $\nu_{max.}$(KBr) 3 540 and 3 415 (OH), 3 010 (6-ene), and 1 705 cm^{-1} (CO); δ(C_5D_5N) 0.87 (s, 18-H_3), 1.26 (s, 19-H_3), 1.42 (d, *J ca.* 6.5 Hz, 21-H_3), 3.38 (d, *J ca.* 13 Hz, 12β-H), 4.08 (m, *w* 7 Hz, 20β-H), 5.38 (s, 17-OH), 5.60 (m, 6,7-H_2), and 5.95 (d, *J ca.* 6 Hz, 20α-OH); *m/z* 346 (M^+), 328, 313, 301, 283, 275, 257, 225, and 197.

3α,17,20α-*Trihydroxy*-5β-*pregnan*-11-*one* (**18**).—17,20α-Dihydroxy-5β-pregn-6-ene-3,11-dione (**11**) (2.4 mg) in dioxane (1 ml) was hydrogenated over 10% Pd–C (3.0 mg) for 6 h. After filtration of the catalyst and evaporation of the solvent under N_2, the product, a glass (2.2 mg), was dissolved in methanol (0.6 ml), treated with sodium borohydride (3.0 mg), and stored overnight at room temperature. Dilution with water followed by isolation with ethyl acetate and purification from ether gave 3α,17,20α-trihydroxy-5β-pregnan-11-one (**18**) (1.0 mg), m.p. 189—191 °C (lit.,[20] m.p. 192—193 °C and 208—210 °C); δ(C_5D_5N) 0.80 (s, 18-H_3), 1.34 (s, 19-H_3), 1.40 (d, *J ca.* 6.5 Hz, 21-H_3), 3.26 (d, *J ca.* 12 Hz, 12β-H), *ca.* 3.80 (m, 3α-H) overlapping 4.04 (m, *w* 7 Hz, 20β-H), 5.13 (s, 17-OH), and 5.84 (overlapping d appearing as a t; OH,OH).

3,3-*Ethylenedioxy*-17,20α-*isopropylidenedioxypregn*-5-*en*-11-*one* (**14**).—A solution of 17,20α-isopropylidenedioxypregn-4-ene-3,11-dione[11] (**13**) (324 mg) in 2-ethyl-2-methyl-1,3-dioxolane (6.0 ml) with toluene-*p*-sulphonic acid (6.0 mg) was slowly distilled under a gentle flow of nitrogen until the volume had been reduced to 1.5 ml. After cooling, a solution of sodium carbonate (30 mg) in water (7.5 ml) was added. The product was isolated with ethyl acetate, and chromatographed on alumina. Fractions eluted with 5% diethyl ether in benzene afforded a colourless gum which gave 3,3-ethylenedioxy-17,20α-isopropylidenedioxypregn-5-en-11-one (**14**) (57.1%) (206 mg), m.p. 80—90 °C from aqueous methanol; $\nu_{max.}$(KBr) 1 700 (CO) and 1 100 cm^{-1}; δ($CDCl_3$) 0.84 (s, 18-H_3), 1.23 (s, 19-H_3), 1.34 (d, *J* 6 Hz, 21-Me), 1.40 (s, acetonide-H_6), 3.91 (s, OCH_2CH_2O), 4.20 (q, *J* 8 Hz, 20β-H), and 5.30 (s, 6 H).

3,3-*Ethylenedioxy*-17,20α-*isopropylidenedioxy*-5β-*pregnane*-6β,11β-*diol* (**15a**).—3,3-Ethylenedioxy-17,20α-isopropylidenedioxypregn-5-en-11-one (**14**) (184 mg) in dry tetrahydrofuran (6.0 ml) under nitrogen at 0 °C was treated dropwise with 1M-'borane–tetrahydrofuran stabilised with sodium borohydride' (Aldrich Chemical Co. Ltd.) during 15 min. After being stirred at room temperature for 2 h, the mixture was cooled to 0 °C and treated with 10M-sodium hydroxide solution (3.3 ml) followed by 30% hydrogen peroxide (3.3 ml), then left

overnight. The product was isolated with ethyl acetate. Purification from this solvent and n-hexane gave the 6β,11β-diol (**15a**) (165 mg, 85.7%), m.p. 105—111 °C; $\nu_{max.}$(KBr) 3 485 (OH), 1 109, 1 080, and 1 040 cm^{-1}; δ(C_5D_5N) 1.32 and 1.36 (s, s, 18- and 19-H_3), 1.44, 1.50, and 1.55 (s,s,s, 21-H_3 and acetonide-H_6), 3.91 (s, OCH_2CH_2O), 4.28 (m, *w* 12 Hz, 20-H), 4.66 (s, *w* 11α-H), and 5.22 (m) and 5.74 (m) (OH,OH).

3,3-*Ethylenedioxy*-17,20α-*isopropylidenedioxy*-5β-*pregnane*-6β,11β-*diol* 6-*Acetate* (**15d**).—The 6β,11β-diol (**15a**) (155 mg) in pyridine (4.0 ml) and acetic anhydride (2.0 ml) were poured into iced water after 17 h at room temperature. The precipitate (105 mg) (m.p. 91—95 °C) was collected and purified by h.p.l.c. on a μ-Porasil column. The more polar of two major peaks gave the 6-acetate (43 mg, 25.4%) as a glass; $\nu_{max.}$(KBr) 3 490 (OH), 1 730 and 1 250 (acetoxy), 1 215 and 1 005 cm^{-1} (acetonide); δ(C_5D_5N) 1.34 (s, 18-H_3), 1.44 and 1.48 (overlapping d and s,s, 21-H_3 + acetonide-H_6), 1.72 (s, 19-H_3), 2.04 (s, OAc), 3.86 (s, OCH_2CH_2O), 4.24 (m, *w* 6 Hz, 20β-H), 4.63 (s, *w* 8 Hz, 11α-H), 4.85 (s, *w* 7 Hz, 6α-H), and 5.37 (s, 11β-OH) (Found: C, 68.6; H, 8.8. $C_{28}H_{44}O_7$ requires C, 68.3; H, 9.0%).

3α,6β,17,20α-*Tetrahydroxy*-5β-*pregnan*-11-*one* 6-*Hemisuccinate* (**4**).—The 6-acetate (**15d**) (21.1 mg) in dichloromethane (0.25 ml) was added to a stirred mixture of pyridinium chlorochromate (12.5 mg) and anhydrous sodium acetate (1.25 mg) in dichloromethane (0.085 ml) and stirring was continued for 2 h. After dilution with anhydrous ether, the mixture was filtered through a small pad of Florisil, which was washed with more ether and dichloromethane. The filtrate and washings were evaporated and the residual brown gum, in ethyl acetate, was filtered through a short column of Florisil to give 6β-acetoxy-3,3-ethylenedioxy-17,20α-isopropylidenedioxy-5β-pregnan-11-one (**15b**) as a colourless gum (18.7 mg); $\nu_{max.}$(KBr) 1 730 (OAc), 1 700 (CO), and 1 250 cm^{-1} (OAc).

The above gum, in ethanol (1.8 ml) with potassium hydroxide (24 mg) in water (0.24 ml) was heated under reflux for 3 h and isolated with ethyl acetate to give the 6β-ol (**15c**) as a pale yellow glass (16.9 mg); $\nu_{max.}$(KBr) 3 450 (OH) and 1 700 cm^{-1} (CO).

This glass (15.0 mg) in dry pyridine (0.17 ml) with freshly recrystallised succinic anhydride (32.0 mg) was heated on the steam bath for 5.5 h, then evaporated to dryness under reduced pressure. The product was dissolved in 50% methanol and after 30 min diluted with water and isolated with ether.

To remove the protecting groups the crude 6-hemisuccinate in 60% acetic acid (5.0 ml) was stored at room temperature (65 h), then the solvent was evaporated to give 6β,17,20α-trihydroxy-5β-pregnane-3,11-dione 6-hemisuccinate (**16**) as a gum (16.5 mg); $\nu_{max.}$(KBr) 3 440 (OH), 1 705 (CO) (shoulder at 1 730, succinate), and 1 170 cm^{-1} (succinate). This was dissolved in methanol (3.0 ml), treated with sodium borohydride (16.8 mg), and stored at room temperature (18 h). Further borohydride (16.8 mg) was added and after 24 h the reaction was stopped by the addition of dilute acetic acid. Isolation with ethyl acetate gave a pale yellow gum (11.4 mg). This was purified by partition chromatography on a column of acid-washed Celite 535 (2.0 g) employing t-butyl alcohol (20 ml), toluene (80 ml), acetic acid (30 ml), and water (70 ml) as the solvent system. Fractions of 2.0 ml were collected, nos. 17—28 giving 3α,6β,17,20α-tetrahydroxy-5β-pregnan-11-one 6-hemisuccinate (**4**) as a solid [1.6 mg, 8% from (**15d**)], m.p. 117—120 °C. The i.r. and ^1H n.m.r. spectra were identical with those of the previous sample (see p. 1209). The ^{13}C n.m.r. spectra were likewise identical. ^{13}C Resonances (δ in p.p.m. relative to $SiMe_4$) with assignments were as follows. δ(C_5D_5N) 221.1 (C-11), 172.2 (CO, succinyl), 84.9 (C-17), 75.1 (C-6), 71.4 (C-3), 70.4 (C-20), 51.6 (C-14), 50.4 (C-13), 34.8 (C-10), 25.6 (C-19), 23.5 (C-15), 19.2 (C-21), and 15.6 (C-18). Selective decoupling by irradiation at the resonance frequency of the C-19 protons (δ 1.64) gave enhancement of the δ 25.6 band and thus confirmed that it was due to C-19.

Alternative Route to 17,20α-*Dihydroxy*-5β-*pregn*-6-*ene*-3,11-*dione* (**11**).—6β-Hydroxy-3,3-ethylenedioxy-17,20α-isopropylidenedioxy-5β-pregnan-11-one (**15c**) (9.3 mg) in dry pyridine (0.1 ml) at 0 °C was treated with phosphorus oxychloride (3 × 5 μl at 5 min intervals) with stirring. The mixture was allowed to attain room temperature and after storage overnight was chilled in ice before being treated with ice followed by water. The product (7.4 mg) isolated with ethyl acetate was left in 60% acetic acid at room temperature for 93 h before being evaporated to dryness. The product was purified by partition chromatography on Celite (2.0 g) employing the Bush B2 system. 2.0-ml Fractions were collected, nos. 5—9 giving the 6-ene (**11**) (1.5 mg, 20.9%), m.p. 200—212 °C (methanol), identical in all respects with that prepared by the route described previously.

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