18-Substituted Steroids. Part 11.[1] Synthesis of 3β,16α,18-Trihydroxyandrost-5-en-17-one, a Neonatal Urinary Metabolite, and the 3,16,18-Triacetate of Its 16β-Epimer

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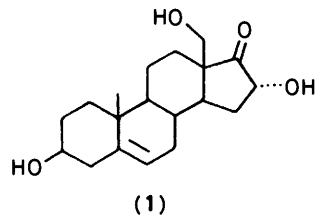
3β,16α,18-Trihydroxyandrost-5-en-17-one and the triacetate of its 16β-epimer have been synthesized from 3β-hydroxypregna-5,16-dien-20-one *via* the 'hypoiodite' reaction of the derived 3β-acetoxy-16α,17α-epoxypregn-5-en-20-ols to functionalize C-18, followed by controlled opening of the epoxide ring by reagents chosen to give either 3β,16α,18- or 3β,16β,18-triacetoxy-17α-hydroxypregn-5-en-20-one. Reduction of the 20-oxo group and oxidative cleavage of the C(17)–C(20) bond produced the corresponding 3β,16,18-triacetoxyandrost-5-en-17-one. 3β,16α,18-Trihydroxyandrost-5-en-17-one was obtained by deacetylation of its triacetate, but the 16β-epimer rearranged on hydrolysis to give 3β,17β,18-trihydroxyandrost-5-en-16-one. The androst-5-ene-3β,16,17β,18-tetraols were also prepared.

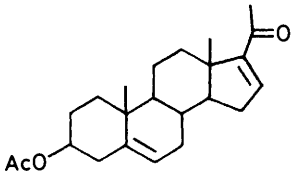
Steroid metabolism in the human foetus and during the first weeks after birth differs markedly from that in later life.[2–5] Among unusual metabolites found in the newborn, Shackleton and Taylor[6] reported the urinary excretion of relatively large amounts (700—2 000 μg per 24 h) of the novel 3β,16,18-trihydroxyandrost-5-en-17-one. Evidence as to the configuration at C-16 was not conclusive, although the 16α-configuration appeared more probable. Mass spectrometry, while establishing the 16- and 18-positions of hydroxylation, could not distinguish between isomers at C-16; the corresponding compounds lacking C-18 substitution (3β,16α- and 3β,16β-dihydroxyandrost-5-en-17-one) have been observed to give very similar fragmentation patterns.[6]

Our own n.m.r. study of the triacetate obtained from natural 3β,16,18-trihydroxyandrost-5-en-17-one, available in moderately pure form in only sub-milligram quantity, was indicative of the 16α-configuration but was not regarded as decisive in view of the high noise level of the spectrum and the absence of knowledge of any possible effects of the 18-hydroxy group on the conformational or spectral features of ring D.

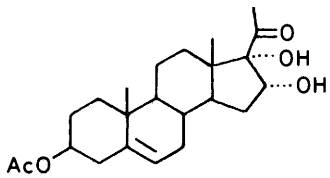
We now report a chemical synthesis of 3β,16α,18-trihydroxyandrost-5-en-17-one (**1**), which proved to be identical with the natural material. The 16β-isomer, albeit only as its triacetate, was synthesized for comparison.



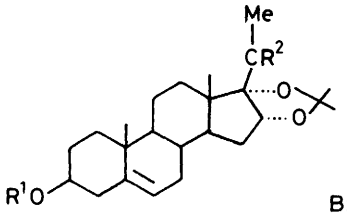
(**1**)



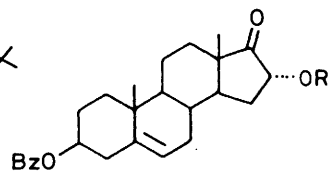
(**2**)



(**3**)



(**4**) R^1 = Ac, R^2 = O
(**5**) R^1 = H, R^2 = H, OH
(**6**) R^1 = Bz, R^2 = H, OH
(**7**) R^1 = Bz, R^2 = H, OBz



(**8**) a; R = H
b; R = Bz

Results and Discussion

Our strategy for both syntheses was to convert 3β-acetoxypregna-5,16-dien-20-one (**2**) into a suitably protected 16,17-disubstituted pregnan-20-ol, to permit functionalization at C-18 *via* the 'hypoiodite' reaction.[7,8] The pregnane side chain would finally be degraded to the required androstan-17-one. A similar route was recently reported for the synthesis of 18-hydroxyoestrone[9] but without the complication of a substituent at C-16. As a direct method for introducing the 16α-hydroxy group, the 16-en-20-one (**2**) was initially hydroxylated by permanganate to the known 16α,17α-diol (**3**)[10,11] which was protected as its acetonide (**4**) before reduction of the 20-oxo function. This reduction could not be achieved by complex hydride without simultaneous deacetylation at C-3, since forcing conditions were required. Selective benzoylation (but not acetylation) of the resulting 3,20-diol (**5**) proved possible at C-3. The product (**6**) was a separable mixture of isomers at C-20, in which the 20β-alcohol was predominant. Configurations were assigned by use of pyridine-induced shifts of methyl proton signals[12] (Table).

When the 3β-benzoyloxy-20-hydroxy compound (**6**) (unseparated isomers) was subjected to the 'hypoiodite' reaction [$Pb(OAc)_4$–I_2–*hν*] the crude product was a complex mixture of compounds, mainly of low polarity. There was no evidence of any attack at C-18 (n.m.r.). Only the most polar component could readily be isolated in pure condition by t.l.c. or h.p.l.c., though in low yield. Its i.r. spectrum ($\nu_{max.}$ 1 745 cm^{-1}) suggested that degradation to an androstan-17-one had

Table. Pyridine-induced chemical shifts [$\Delta = \delta(C_5D_5N) - \delta(CDCl_3)$] in ^1H n.m.r. spectra of pregnan-20-ols (*cf.* ref. 12)

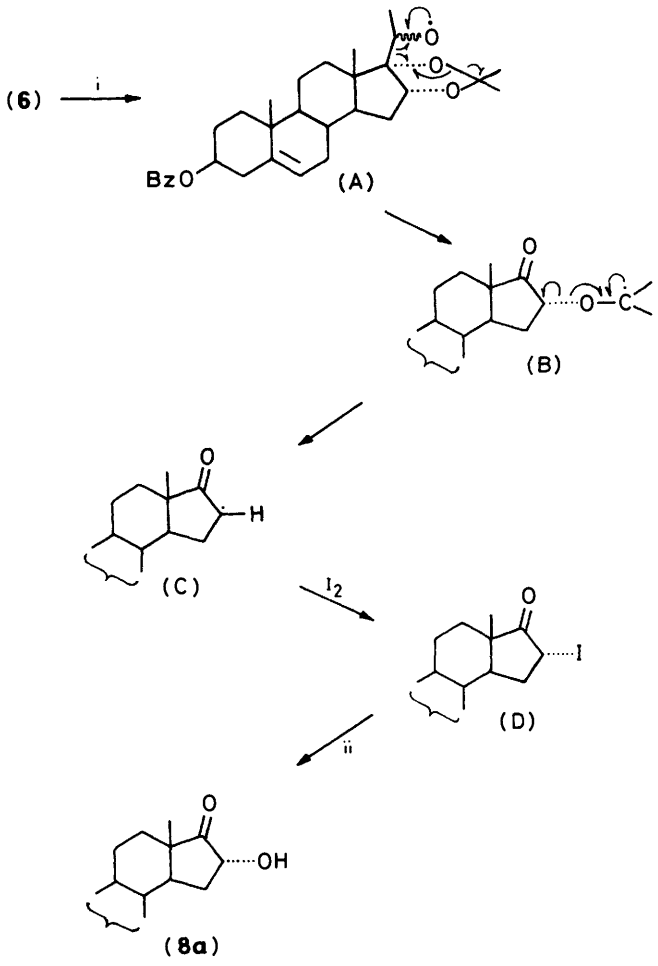
	18-H_3/H_2			20-H			21-H_3			16-H			Acetonide-CH_3		
Compound	$CDCl_3$ (δ)	C_5D_5N (δ)	Δ	$CDCl_3$ (δ)	C_5D_5N (δ)	Δ	$CDCl_3$ (δ)	C_5D_5N (δ)	Δ	$CDCl_3$ (δ)	C_5D_5N (δ)	Δ	$CDCl_3$ (δ)	C_5D_5N (δ)	Δ
3β-Benzoyloxy-16α,17α-isopropylidenedioxy-pregn-5-en-20-ols (**6**)															
20β-OH	0.92	1.24	0.32	4.10	4.40	0.30	1.335	1.55	0.215	4.52	5.04	0.52	1.50 1.54	1.52 1.64	0.02 0.10
20α-OH	0.89	0.90	0.01	3.98	4.32	0.34	1.27	1.53	0.26	4.72	5.20	0.48	1.52 1.58	1.72 1.92	0.20 0.34
3β-Acetoxy-16α,17α-epoxypregn-5-en-20-ols (**11**)															
20β-OH	0.90	0.96	0.06	4.34	4.68	0.34	1.11	1.29	0.18	3.32	3.20	−0.12			
20α-OH	0.90	0.88	−0.02	4.20	4.56	0.36	1.33	1.55	0.22	3.50	3.86	0.36			
3β,16β,18-Triacetoxy-17α-hydroxypregn-5-en-20-ols (**28**)															
20β-OH	4.32	4.74	0.42	4.20	4.92	0.72	1.14	1.51	0.37	4.82	5.34	0.52			
20α-OH	4.30	4.64	0.34	3.94	4.90	0.96	1.30	1.70	0.40	4.92	5.66	0.74			

occurred. The product appeared from its n.m.r. spectrum to be 3β,16α-dihydroxyandrost-5-en-17-one 3-benzoate (**8a**); this structure was confirmed by conversion into the 3,16-dibenzoate (**8b**), which proved to be identical with a sample prepared from the known 3β,16α-dihydroxyandrost-5-en-17-one. The mechanism of formation of the 17-ketone, with loss of the acetonide, probably involves rupture of the C(17)–C(20) bond in the 20-oxy radical (Scheme 1) with participation of electrons in the acetonide ring. An alternative hydrogen abstraction by the 20-oxy radical from the nearer of the acetonide methyl groups cannot be ruled out, although it would involve an unusual seven-membered cyclic transition state.

Attention was next turned to the 16α,17α-epoxypregnan-20-ol (**11**), available as a mixture of isomers at C-20 (see Experimental and Table), as an intermediate suitable for functionalization of C-18. The epoxide ring is surprisingly stable under the conditions of the hypoiodite reaction, which had been reported[13,14] to give 18-acetoxy-18,20-epoxy products derived from the 18,20-epoxy-18-iodo-system when sufficient of the reagent was used to ensure bifunctionality at C-18.

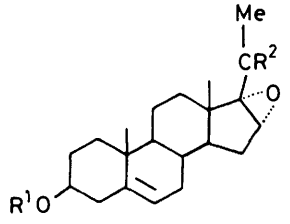
By using smaller proportions of reagents in the hypoiodite reaction we were able to limit the extent of attack at C-18 to obtain the 18-iodopregnan-20-ol[15] (**12**). Oxidation of the crude product mixture gave the 18-iodo-20-ketone (**13**), converted by Ag^+-assisted hydrolysis at C-18 into the required 16α,17α-epoxy-18-hydroxypregn-5-en-20-one (**14**), which was isolated by preparative h.p.l.c. The hydrolysis of (**13**) was unusually slow for such an iodo-ketone. We attribute this to the difficulty of participation by the C-20 oxygen atom to form an intermediate bridge between C-18 and C-20 (see below). From the complex mixture of products it also proved possible to isolate in lower yields the known 18-acetoxy-18,20-epoxy derivative (**17**), as well as some recovered 16α,17α-epoxy-20-ketone (**10**). Clearly bifunctional attack at C-18 occurs at a rate comparable with the first hydrogen abstraction, making choice of reaction conditions critical to optimise the yield of the required product.

A further minor product was characterised as 3β,18-diacetoxy-16α,17α; 18,20-diepoxypregn-5-en-7-one (**18**), formed by an allylic oxidation at C-7.[16] Confirmation of this unusual variation on the hypoiodite reaction was obtained when 3β-acetoxycholest-5-en-7-one was found to be formed in low yield from cholesteryl acetate under the same conditions.

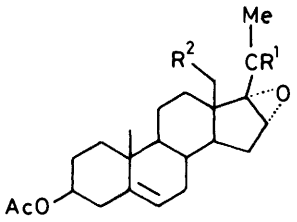


Scheme 1. Possible pathway for the formation of the 16α-hydroxy-17-one (**8a**). i, $Pb(OAc)_4$–I_2–*hν* in cyclohexane. ii, Ag(OAc)–water–dioxane

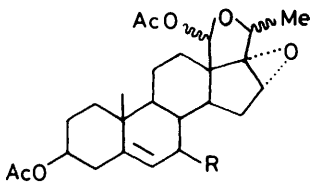
The required 16α,17α-epoxy-18-hydroxypregn-5-en-20-one (**14**) was unusual in existing as the hydroxy-ketone rather than



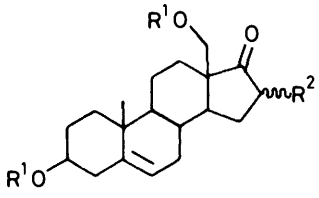
(9) $R^1 = H$, $R^2 = O$
(10) $R^1 = Ac$, $R^2 = O$
(11) $R^1 = Ac$, $R^2 = H, OH$



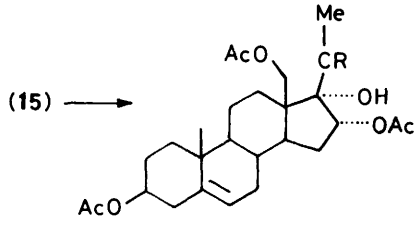
(12) $R^1 = H, OH$, $R^2 = I$
(13) $R^1 = O$, $R^2 = I$
(14) $R^1 = O$, $R^2 = OH$
(15) $R^1 = O$, $R^2 = OAc$
(16) $R^1 = NOH$, $R^2 = OAc$



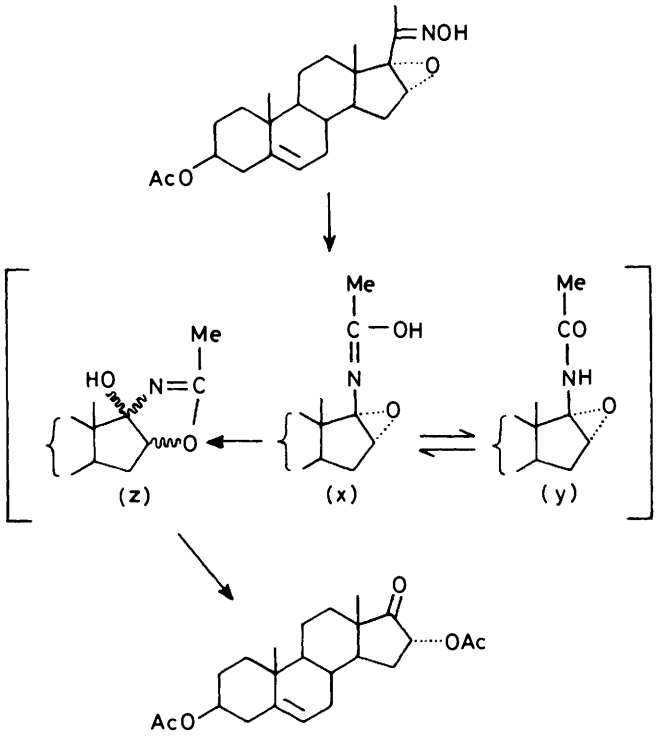
(17) $R = H_2$
(18) $R = O$



(19) $R^1 = Ac$, $R^2 = \alpha$-OAc
(20) $R^1 = Ac$, $R^2 = \alpha$-OH
(21) $R^1 = H$, $R^2 = \beta$-OH
(22) $R^1 = Ac$, $R^2 = \beta$-OAc



(23) $R = NNPh_2$
(24) $R = NNHCO_2Et$
(25) $R = O$
(26) $R = H, OH$



Scheme 2. (*cf.* ref. 18)

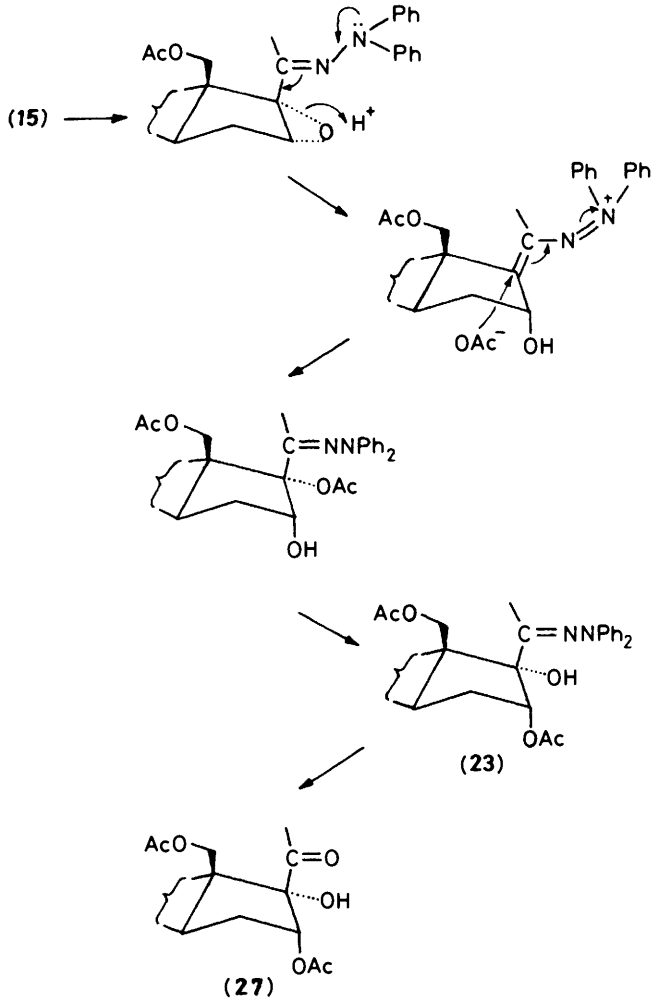
as the 18,20-hemiacetal, commonly observed for other 18-hydroxypregnan-20-ones.[1,17] The i.r. and n.m.r. spectra clearly showed the features associated with the open hydroxy-ketone formulation (see Experimental). Acetylation (Ac_2O–pyridine) converted the compound smoothly into the 18-acetate (**15**), in contrast with the forcing conditions necessary to obtain the 18-acetoxy-ketone from the hemiacetal form of 18-hydroxyprogesterone and related compounds.[17] Inspection of a Dreiding model shows that the change in geometry of ring D resulting from the presence of the 16α,17α-epoxide tilts the side chain away from C-18, so that hemiacetal formation becomes relatively unfavourable. The slow hydrolysis of the iodo-ketone (**13**) mentioned above probably has a similar explanation.

Having obtained 3β,18-diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) it was necessary to degrade the pregnane side chain. Beckmann rearrangement[18] of the 20-oxime of 3β-acetoxy-16α,17α-epoxypregn-5-en-20-one (**10**) is reported to give 3β,16α-diacetoxyandrost-5-en-17-one directly; the precise mechanism (Scheme 2) for the transfer of the acetyl group comprising the pregnan-20-one side chain to the 16α-oxygen substituent is not clear, but was presumed[18] to involve an intramolecular rearrangement of the intermediate 17β-acetamido-16α,17α-epoxide. Our first attempt to apply this procedure to the degradation of the 18-acetoxy compound (**15**) led to a product of unexpected structure, derived from the 20-oxime, which will be described elsewhere.[19]

On further investigation we traced the source of the problem to the use of reflux conditions in order to force the rather reluctant oximation, which was probably subject to steric hindrance by the 18-acetoxy substituent.[20] The 20-oxime (**16**) was subsequently obtained by prolonged reaction of the ketone (**15**) with hydroxylamine at room temperature, and was found to undergo a normal Beckmann rearrangement to give 3β,16α,18-triacetoxyandrost-5-en-17-one (**19**) in acceptable yield.

In the meantime we explored other ways of degrading the 16,17-epoxy-ketone (**15**) to the required androstan-17-one derivative.

Since it was necessary to use the 16α,17α-epoxide ring as the source of a 16α-oxygen function (the abnormal mode of epoxide opening), attention was turned to the reaction of the 20-oxo group with a suitable hydrazine derivative. 1,1-Diphenylhydrazine in acetic acid formed the diphenylhydrazone,[21] which provides the necessary 'electron push' for the abnormal opening of the epoxide ring at C-17 through the mechanism illustrated in Scheme 3. The product was the 16α-acetoxy-17α-hydroxy-20-one diphenylhydrazone (**23**), presumably resulting from ester migration in an initially formed 17α-acetate (Scheme 3). Many methods (indicated in the caption to scheme 3) were explored for the regeneration of the 20-oxo function from the diphenylhydrazone, but the only two to be reasonably successful were the old-fashioned transfers of the diphenylhydrazone to either pyruvic acid[22] or *p*-hydroxybenzaldehyde,[23] followed by an alkaline wash. All reactions involving the diphenylhydrazone were complicated by the formation of a series of deeply coloured by-products (t.l.c.). Superior results were obtained subsequently



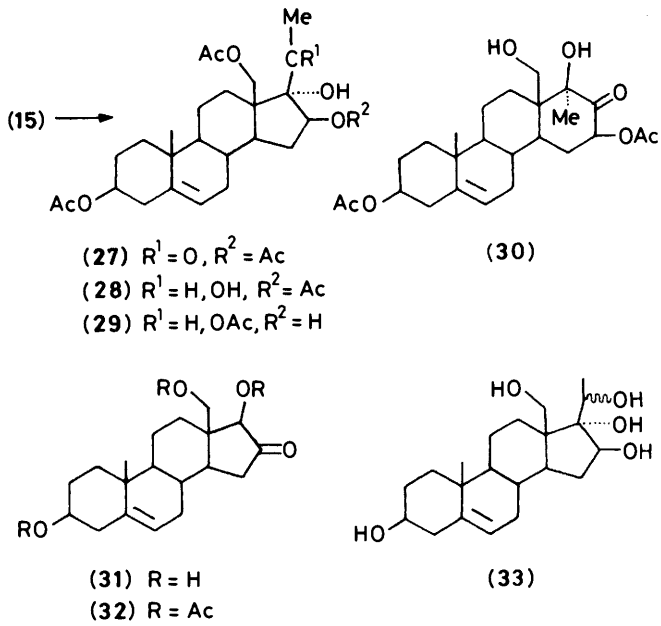
Scheme 3. (*cf.* ref. 21) i, *N,N*-Diphenylhydrazine in glacial acetic acid. D Ring only shown. *Reagents which failed to cleave the diphenylhydrazone efficiently.* Nitrosonium tetrafluoroborate, G. A. Olah and T. L. Ho, *Synthesis*, 1976, 610; exchange reaction with acetone, S. A. Maynez, L. Pelavin, and G. Erker, *J. Org. Chem.*, 1975, **40**, 3302; benzeneseleninic anhydride, D. H. R. Barton, D. J. Lester, and S. V. Ley, *J. Chem. Soc., Chem. Commun.*, 1977, 445; hydrogen peroxide–potassium carbonate, J. Jiricny, D. M. Oree, and C. B. Reese, *Synthesis*, 1978, 919; pyridinium chlorochromate–hydrogen peroxide, J. Drabowicz, *Synthesis*, 1980, 125; aqueous sodium periodate, E. Corey and D. Enders, *Tetrahedron Lett.*, 1976, 3; aqueous copper(I) acetate, E. Corey and D. Enders, *Tetrahedron Lett.*, 1976, 3667; and cerium(IV) ammonium nitrate, J. W. Bird and G. M. Diaper, *Can. J. Chem.*, 1969, **47**, 145

by similar use of ethoxycarbonylhydrazine(ethyl carbazate),[22] which converted 3,18-diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) into the 20-ethoxycarbonylhydrazone (**24**) of 3β,16α,18-triacetoxy-17α-hydroxypregn-5-en-20-one (**25**). Cleavage of the hydrazone derivative (**24**) with *p*-hydroxybenzaldehyde[23] cleanly regenerated the 20-oxo function.

Reduction of the 20-oxo group with sodium borohydride then gave 3β,16α,18-triacetoxypregn-5-ene-17α,20-diol (**26**), which appeared (n.m.r. and h.p.l.c.) to be essentially a single isomer at C-20, characterised as the 20β alcohol by pyridine-induced chemical shifts.[12] Degradation of the 17α,20-diol (**26**) to the androstan-17-one (**19**) by periodic acid[24] was very slow, probably because the 16α- and 18-acetoxy groups hinder the formation of an intermediate cyclic 17α,20-periodate. 3β,16α,18-Triacetoxyandrost-5-en-17-one (**19**) was obtained, but was accompanied by another (less polar) product which has not been identified. A more satisfactory cleavage of the side-chain was accomplished by the use of lead tetra-acetate.[25] The resulting 3,16,18-triacetate (**19**) had the expected n.m.r. characteristics, including a multiplet at δ 5.46 for the 16β-proton, with a profile characteristic of the 16α-acetoxy-17-oxo system.

Hydrolysis of the triacetate was achieved both enzymically (digestive juice of *Helix pomatia*[26,27]) and by acidic catalysis,[28] to give 3β,16α,18-trihydroxyandrost-5-en-17-one (**1**), which proved to be identical with the material of urinary origin. The comparison was made by g.c.–mass spectral analysis of the methoxyimino-trimethylsilyl ether (MO-TMS) derivative, and by matching of the ^{1}H n.m.r. spectral features of triacetates of natural and synthetic materials.

In an attempt to obtain 3β,16β,18-trihydroxyandrost-5-en-17-one (**21**) for comparison, the epoxide ring in 3β,18-diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) was opened in the normal way by the use of acetic acid–sulphuric acid[29] to give 3β,16β,18-triacetoxy-17α-hydroxypregn-5-en-20-one (**27**). A small amount of D-homo-steroid (**30**) (see Experimental section) was also



isolated. The 20-oxo compound (**27**) was then reduced with sodium borohydride to the mixed 20-hydroxy derivatives (**28**) (Table), which were cleaved with lead tetra-acetate to give 3β,16β,18-triacetoxyandrost-5-en-17-one (**22**). The n.m.r. spectrum showed the expected differences from that of the 16α-isomer (**19**), notably in the signal for the proton at C-16 (a triplet at δ 5.02). The mass spectra of the two C-16 epimeric triacetates, however, were almost indistinguishable.

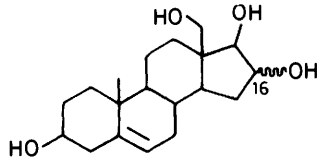
16β-Hydroxyandrostan-17-ones are known to be very unstable to either acid or base, being readily converted into the isomeric 17β-hydroxyandrostan-16-ones by intramolecular hydride transfer.[30] We therefore attempted to obtain the 16β-hydroxyandrostan-17-one (**21**) from the triacetate (**22**) by enzymic hydrolysis.[26,27] The hydrolysis occurred exceptionally slowly compared with the 16α-epimer, and the product was found to have undergone rearrangement to give 3β,17β,18-trihydroxyandrost-5-en-16-one (**31**). Reacetylation gave the triacetate (**32**), which differed from the original triacetate (**22**). Despite numerous attempts with different enzymes[31,32] and media, either the rearrangement could not be avoided or the substrate remained unchanged.

We therefore explored an alternative route from 3β,16β,18-triacetoxy-17α-hydroxypregn-5-en-20-one (**27**), having first established that the 18-deoxy analogue could be converted into 3β,16β-dihydroxyandrost-5-en-17-one by the same sequence. Reduction at C-20 with borohydride was followed by alkaline hydrolysis of the ester groups to give the pregn-5-ene-3β,16β,17α,18,20-pentaols (**33**). Careful cleavage of the side chain by the action of periodate in neutral solution was expected to give the required 3β,16β,18-trihydroxy compounds (**21**), but the pentaols (**33**) were recovered unchanged after treatment with sodium metaperiodate in aqueous methanol under the neutral conditions required to permit survival of the 16β-hydroxy-17-ketone.

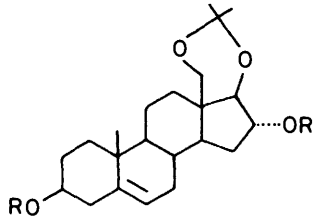
It is apparent from the outcome of the enzymic hydrolysis that the 18-hydroxy group enhances the tendency of a 16β-hydroxy-17-ketone to isomerise. It seems most unlikely that the 3β,16β,18-trihydroxy compound (**21**) could survive under physiological conditions or during extraction [33] even were it ever to arise naturally. This finding further strengthens the assignment of the 16α-configuration to the urinary 3β,16,18-trihydroxy compound (**1**).

The corresponding 3β,16,17β,18-tetraols (16α and 16β isomers) were obtained by sodium borohydride reduction of the corresponding 17-ketones. 3β,16α,18-Trihydroxyandrost-5-en-17-one was reduced directly, whereas for the 16β isomer, the triacetate was first reduced at C-17 and then hydrolysed. For both isomers the 17β-alcohol was expected to be the main product, as borohydride reductions are known to attack the 17-oxo group preferentially from the α-face, although the possibility of an abnormal directing effect of the substituents at C-16 and C-18 could not be discounted.

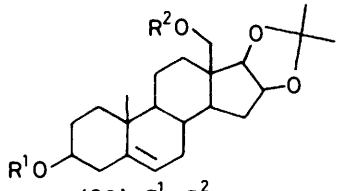
Acetonide formation confirmed the 17β configuration of both tetraols. The 3β, 16α, 17β, 18-tetraol (**34**) in 2,2-dimethoxypropane [34] and dimethylformamide containing a catalytic



(**34**) 16α-OH
(**35**) 16β-OH



(**36**) R = H
(**37**) R = Ac



(**38**) $R^1 = R^2 = H$
(**39**) $R^1 = R^2 = Ac$
(**40**) $R^1 = Ac, R^2 = H$

amount of toluene-*p*- sulphonic acid at 80 °C was found to form an acetonide (**36**) between the 17β-and 18-hydroxy groups because the bridging of 16α and 17β would require excessive strain. Acetylation of the derivative (**36**) gave the 3β,16α-diacetate (**37**), confirmed from the ^1H n.m.r. spectrum which showed that the 16β- and 3α-proton signals had each moved downfield by nearly 1 p.p.m. as a result of acetylation.

In the case of the 16β-tetraol (**37**) the 16β,17β-acetonide (**38**) was very easily prepared using the same conditions. On acetylation of the acetonide (**38**), the C_{18}-methylene and the 3α-proton signals in the n.m.r. spectrum moved downfield, while those for 17α-H and 16α-H were unaffected. Isolation of 3β-acetoxy-18-hydroxy-16β,17β-isopropylidenedioxyandrost-5-ene (**40**) as a by-product from the acetylation of the acetonide (**38**) is attributed to the steric hindrance from the isopropylidene-group to acetylation at C-18.

Experimental

M.p.s were determined on a Reichert melting point microscope. I.r. spectra were determined for potassium bromide discs. N.m.r. spectra were determined by Dr. R. D. Farrant at 100 MHz for solutions in deuteriochloroform unless otherwise stated, with tetramethylsilane as internal standard. Nuclear Overhauser studies were carried out at Queen Mary College on the University of London Intercollegiate Research Service (ULIRS) Bruker WH 400 MHz F.T. n.m.r. spectrometer. Unless otherwise indicated mass spectra were obtained by electron impact on the ULIRS Kratos MS25 instrument at Queen Elizabeth College. Mass spectra indicated as 'F.A.B.' (fast atom bombardment) were obtained by bombardment with argon in the ULIRS VG-Analytical Ltd. ZAB 1F instrument at the School of Pharmacy. All solvents were purified before use.[35] 'Hexane' refers to the light petroleum fraction of boiling range 60—80 °C and ether refers to diethyl ether. Column chromatography was carried out on Alumina (Type H, Leoparte Industries). 'Deactivated alumina' refers to alumina which had been treated with 5% by weight of aqueous 10% acetic acid. T.l.c. was carried out on Kieselgel $60PF_{254+366}$. Analytical h.p.l.c. was on Waters Associates 3.9 mm i.d. × 30 cm μ Porasil and μ Bondapak C_{18} columns. Preparative h.p.l.c. was carried out on a Waters Associates Prep-LC/system-500. In both cases a differential refractometer was used for detection. Elution characteristics are given as capacity factor, $k' = (V_1 - V_0)/V_1$, where V_0 = column volume of solvent and V_1 = column volumes required to elute the compound. The digestive juice of *Helix pomatia* was supplied by Uniscience Ltd.

3β-Acetoxy-16α,17α-dihydroxypregn-5-en-20-one (**3**) was prepared from 3β-acetoxypregna-5,16-dien-20-one (16-dehydropregnenolone acetate) (**2**) as described [11] and protected as its 16,17-acetonide (**4**) before reduction of the C-20 carbonyl group.[36]

16α,17α-Isopropylidenedioxypregn-5-ene-3β,20-diols (**5**).—3β-Acetoxy-16α,17α-isopropylidenedioxypregn-5,20-one (**4**) (500 mg, 1.163 mmol) was added to a mixture of lithium aluminium hydride (365 mg, 9.62 mmol) in dry tetrahydrofuran (10 ml) at 0 °C with stirring for 15 min then the mixture was heated under reflux for 1.5 h. After cooling, excess of reagent was destroyed by adding ethyl acetate, then saturated aqueous magnesium sulphate (4 ml) was added, followed by solid anhydrous magnesium sulphate (5 g). After being stirred for 30 min the mixture was filtered, the solids were washed with more tetrahydrofuran, and the filtrate was dried ($MgSO_4$) and evaporated under reduced pressure. The residual solid (0.497 g) appeared homogeneous on t.l.c.; m.p. 196—198 °C (from acetone); $\nu_{max.}$ 3 480, 1 450, 1 320, 1 245, and 1 050 cm^{-1}; δ 0.90 (s, 18-H_3), 1.04 (s, 19-H_3), 1.35 (d, *J* 7 Hz, 21-H_3), 1.50 and 1.54 (2 s, acetonide methyls), 3.58 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 4.10 (m, $w_{\frac{1}{2}}$ 20 Hz, 20-H), 4.34 (m, $w_{\frac{1}{2}}$ 12 Hz, 16β-H), and 5.38 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H) (Found: C, 73.3; H, 9.8. $C_{24}H_{38}O_4$ requires C, 73.7; H, 9.8%).

3β-Benzoyloxy-16α,17α-isopropylidenedioxypregn-5-en-20-ols (**6**).—The unseparated crude diols (**5**) (1.0 g, 2.56 mmol) in pyridine (5 ml) were treated with benzoyl chloride (1 ml, 8.54

mmol) for 18 h at room temperature. After adding ice–water, the product was extracted with ether, which was washed with saturated aqueous sodium hydrogen carbonate, hydrochloric acid (3M), and water, then dried (K_2CO_3 and $MgSO_4$) and taken to *dryness* under reduced pressure to give a gum (1.36 g). The crude product was chromatographed on deactivated alumina (136 g). Ethyl acetate (3%) in hexane eluted the 3,20-*dibenzoate* (**7**) (0.342 g), m.p. 232—235 °C (acetone); $\nu_{max.}$ 1 715, 1 600w, 1 275, and 720 cm^{-1}; δ 1.04 (s, 18-H_3), 1.08 (s, 19-H_3), 1.50 (d, *J* 8 Hz, 21-H_3), 1.54 (6 H, s, acetonide methyls), 4.54 (d, *J* 6 Hz, 16β-H), 4.82 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.48 (2 H, m, $w_{\frac{1}{2}}$ 20 Hz, 20 and 6-H), and 7.28—7.64 and 7.88—8.12 (aromatic protons) (Found: C, 77.1; H, 7.5. $C_{38}H_{42}O_6$ requires C, 76.8; H, 7.1%). Elution with ethyl acetate (6%) in hexane afforded the 3-monobenzoate (**6**), $\nu_{max.}$ 3 670, 1 720, 1 600w, 1 280, 1 050, 1 070, and 725 cm^{-1}. The two isomers (20α and 20β) were separated in the same solvent, the 20β-*isomer* (0.624 g) being eluted first; m.p. 234—236 °C (prisms, from acetone–hexane); δ 0.92 (s, 18-H_3), 1.09 (s, 19-H_3), 1.335 (d, *J* 7 Hz, 21-H_3), 1.50 and 1.54 (2 s, acetonide methyls), 4.10 (m, $w_{\frac{1}{2}}$ 18 Hz, 20α-H), 4.52 (m, $w_{\frac{1}{2}}$ 12 Hz, 16β-H), 4.82 (m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 5.42 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 7.28—7.64 and 7.92—8.10 (aromatic protons); δ(C_5D_5N) 1.06 (s, 19-H_3), 1.24 (s, 18-H_3), 1.55 (d, *J* 6 Hz, 21-H_3), 1.52 and 1.64 (2 s, acetonide methyls), 4.40 (m, $w_{\frac{1}{2}}$ 20 Hz, 20α-H), 5.04 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.04 (m, $w_{\frac{1}{2}}$ 10 Hz, 16β-H), 5.39 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 7.30—7.66 and 8.08—8.32 (aromatic protons) (Found: C, 75.4; H, 8.3. $C_{31}H_{42}O_5$ requires C, 75.3; H, 8.6%). 20α-*Isomer* (0.0418 g): m.p. 252—256 °C (acetone–hexane); δ 0.89 (s, 18-H_3), 1.08 (s, 19-H_3), 1.27 (d, *J* 6 Hz, 21-H_3), 1.52 and 1.58 (2 s, acetonide methyls), 3.98 (m, $w_{\frac{1}{2}}$ 16 Hz, 20β-H), 4.72 (m, $w_{\frac{1}{2}}$ 10 Hz, 16β-H), 4.82 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.40 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 7.32—7.60 and 7.92—8.12 (aromatic protons); δ(C_5D_5N) 0.90 (s, 18-H_3), 1.06 (s, 19-H_3), 1.53 (d, *J* 6 Hz, 21-H_3), 1.72 and 1.92 (2 s, acetonide methyls), 4.32 (m, $w_{\frac{1}{2}}$ 20 Hz, 20β-H), 4.96 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.20 (m, $w_{\frac{1}{2}}$ 10 Hz, 16β-H), 5.40 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 7.36—7.64 and 8.08—8.36 (aromatic protons) (Found: C, 75.6; H, 8.6. $C_{31}H_{42}O_5$ requires C, 75.3; H, 8.6%). Finally, elution with ethyl acetate afforded a small amount of the 3β,20-diols (**5**).

Attempted Functionalisation of C-18 *using* 3β-*Benzoyloxy*-16α,17α-*isopropylidenedioxypregn*-5-*en*-20-*ols* (**6**).—(*a*) Hypoiodite reaction. The steroid (**6**) (100 mg, 0.202 mmol) in anhydrous cyclohexane (15 ml) was treated with lead tetra-acetate (215.2 mg, 0.486 mmol), calcium carbonate (71.6 mg), and iodine (28.8 mg, 0.113 mmol), and the mixture was then stirred magnetically and heated under reflux while being irradiated from below with two 500 W tungsten lamps. The reaction was stopped after 1 h even though the iodine colour had not been discharged completely. The mixture was cooled to room temperature and filtered. The filtrate was washed with aqueous sodium thiosulphate and water, then dried (K_2CO_3), and the solvent was removed under reduced pressure from a water-bath below 35 °C, to give a pale yellow solid.

(*b*) *Oxidation.* The above residue in acetone (13 ml), was cooled in ice and stirred during dropwise addition of Jones' chromic acid reagent until the orange colour persisted. Aqueous sodium acetate was added and the product was extracted with benzene; the extract was washed with aqueous sodium chloride, dried (K_2CO_3), and taken to dryness under reduced pressure from a water bath at 36 °C.

(*c*) *Silver acetate-assisted solvolysis.* The crude oxidation product (77.3 mg) and silver acetate in 10% aqueous dioxane (6.6 ml) was held at 60—65 °C for 2 h after which the mixture was cooled and filtered. The solvent was removed under reduced pressure from the filtrate to give a pale yellow solid (58 mg), which was a very complex mixture, not resolved by t.l.c. H.p.l.c. separation (30% EtOAc in hexane) showed it to consist of two groups of compounds, obtained as polar (6.3 mg) and less-polar fractions (28 mg). The only component isolated, from the polar fraction, was 3β,16α-*dihydroxyandrost*-5-*en*-17-*one* 3-*benzoate* (**8a**), m.p. 234—237 °C (acetone–hexane); $\nu_{max.}$ 3 460, 1 740, 1 715, 1 605w, 1 280, 1 025, and 725 cm^{-1}; δ 1.00 (s, 19-H_3), 1.11 (s, 18-H_3), 4.38 (t, *J* 4 Hz, 16β-H), 4.86 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.44 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 7.28—7.44 and 7.92—8.20 (aromatic protons) (Found: C, 76.8; H, 7.9. $C_{26}H_{32}O_4$ requires C, 76.4; H, 7.9%). Benzoylation (benzoyl chloride–pyridine) of the monobenzoate (**8a**) and of an authentic sample of 3β,16α-dihydroxyandrost-5-en-17-one gave the 3β,16α-*dibenzoate* (**8b**); the samples were identical in all respects, m.p. 168—171 °C (acetone–hexane); $\nu_{max.}$ 1 755, 1 715, 1700sh, 1 600, 1 280, 1 025, and 720 cm^{-1}; δ 1.08 (s, 19-H_3), 1.12 (s, 18-H_3), 4.86 (m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 5.44 (m, *J* 10 Hz, 6-H), 5.65 (m, $w_{\frac{1}{2}}$ 18 Hz, 16β-H), and 7.32—7.64 and 7.96—8.12 (aromatic protons) (Found: C, 76.6; H, 7.3. $C_{31}H_{36}O_5$ requires C, 76.2; H, 7.4%). The less polar fraction could not be resolved into separate components. It exhibited $\nu_{max.}$ 1 790, 1 740, 1 710, 1 610, 1 280, and 725 cm^{-1}; δ 1.08 and 1.18 (2 s, 18- and 19-H_3), 4.88 (m, $w_{\frac{1}{2}}$ 28 Hz, 3α-H), 5.48 (m, $w_{\frac{1}{2}}$ 12 Hz, 16β-H), 5.98 (d, *J* 2 Hz, H), and 7.30—7.64 and 7.93—8.12 (aromatic protons).

3β-Hydroxy-16α,17α-epoxypregn-5-en-20-one (**9**), was prepared as described by Löken *et al.*[37] and acetylated (acetic anhydride–pyridine) at room temperature to form the 3-acetate (**10**).

3β-*Acetoxy*-16α,17α-*epoxypregn*-5-*en*-20-*ols* (**11**). The 3-acetate (**10**) (1.743 g, 4.68 mmol) in dry tetrahydrofuran (28.6 ml) and dry methanol (8.4 ml) was treated with sodium borohydride (0.336 g, 8.88 mmol) portionwise during 15 min at 5 °C; the mixture was then stirred at the same temperature for 1 h, when t.l.c. showed complete reaction. Acetic acid was added dropwise until the solution was neutral after which the mixture was concentrated under reduced pressure and poured into water. The washed and dried precipitate (1.643 g) crystallised from acetone as needles, m.p. 171—172 °C; $\nu_{max.}$ 3 450, 1 730, 1 250, and 1 040 cm^{-1}; the ^1H n.m.r. spectrum of the crude product showed that it contained the 20β-and 20α-isomers in 3:1 ratio. Pure samples were obtained by h.p.l.c., using 30% ethyl acetate in hexane on a μ-Porasil column [capacity factors (*k'*), 20β, 1.75; 20α, 2.5]. Configurations were assigned by n.m.r. using pyridine-induced shifts.[12] 20β-*Alcohol*: m.p. 168.5—170 °C (from acetone); δ 0.90 (s, 18-H_3), 1.04 (s, 19-H_3), 1.11 (d, *J* 6 Hz, 21-H_3), 2.04 (s, 3-OAc), 3.32 (s, 16β-H), 4.34 (m, $w_{\frac{1}{2}}$ 20 Hz, 20α-H), 4.56 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H); δ(C_5D_5N), 0.96 (s, 18-H_3), 1.00 (s, 19-H_3), 1.29 (d, *J* 6 Hz, 21-H_3), 2.04 (s, 3-OAc), 3.20 (s, 16β-H), 4.48—4.92 (complex, 20α-H and 3α-H), and 5.30 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H) (Found: C, 73.5; H, 9.2. $C_{23}H_{34}O_4$ requires C, 73.7; H, 9.2%). 20α-*Alcohol*: m.p. 219—222 °C (from acetone); δ 0.90 (s, 18-H_3), 1.04 (s, 19-H_3), 1.33 (d, *J* 6 Hz, 21-H_3), 2.04 (s, 3-OAc), 3.50 (s, 16β-H), 3.56 (m, $w_{\frac{1}{2}}$ 25 Hz, 3α-H), 4.20 (m, $w_{\frac{1}{2}}$ 25 Hz, 20β-H), and 5.34 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H); δ(C_5D_5N), 0.88 (s, 18-H_3), 0.98 (s, 19-H_3), 1.55 (d, *J* 6 Hz, 21-H_3), 2.04 (s, 3-OAc), 3.86 (s, 16β-H), 4.32—4.70 (complex, 20β-H and 3α-H), and 5.30 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H) (Found: C, 73.7; H, 9.2. $C_{23}H_{34}O_4$ requires C, 73.7; H, 9.2%).

3β-*Acetoxy*-16α,17α-*epoxy*-18-*hydroxypregn*-5-*en*-20-*one* (**14**).—3β-Acetoxy-16α,17α-epoxypregn-5-en-20-ol (**11**) (mixed isomers) (2 g, 5.34 mmol) in anhydrous cyclohexane (300 ml) containing calcium carbonate (1.8 g) was treated with lead tetra-acetate (5.686 g, 12.83 mmol) and iodine (760.6 mg, 2.99 mmol) and the mixture then stirred magnetically and heated under reflux while being irradiated from below with two 500 W tungsten lamps. When the iodine colour had been discharged (*ca.* 90 m) the mixture was cooled to room temperature and filtered

through a bed of Celite. The filtrate was washed with aqueous sodium thiosulphate and water, dried (K_2CO_3), treated with a few drops of pyridine, and the solvent removed under reduced pressure from a water-bath below 35 °C.

The residual gum in acetone (40 ml) was cooled in ice and stirred during dropwise addition of Jones' chromic acid reagent until the orange colour persisted. Sodium acetate solution (60 ml; 42% aqueous) was then added and the product extracted with benzene. The extract was washed with saturated aqueous sodium chloride, dried (K_2CO_3), and after addition of a few drops of pyridine was evaporated under reduced pressure at 35 °C.

The crude yellow oil containing the iodo ketone (**13**) was dissolved in aqueous 90% dioxane (150 ml) and silver acetate (1.928 g) was added. The mixture was heated *rapidly* with stirring to 65—70 °C and held at this temperature for 24 h. The cooled mixture was filtered and the filtrate was taken to dryness under reduced pressure. Traces of inorganic impurities were removed by dissolution of the residue in ether, filtering of the solution and evaporation of the solvent from the filtrate. The resulting gum was submitted to preparative h.p.l.c. (30% ethyl acetate in hexane) to obtain 3β-*acetoxy*-16α,17α-*epoxy*-18-*hydroxypregn*-5-*en*-20-*one* (**14**) (0.484 g) (*k'* 2.38), m.p. 189—191 °C (plates from acetone–hexane); $\nu_{max.}$ 3 420, 1 730, 1 690, 1 250, and 1 040 cm^{-1}; δ 1.04 (s, 19-H_3), 2.04 (s, 3-OAc), 2.14 (s, 21-H_3), 3.70 (s, 16β-H), 3.45 (d) and 3.86 (t; d on adding D_2O; J_{gem} 12 Hz, $J_{18.OH}$ 11 Hz, 18-H_2), 4.56 (m, $w_{\frac{1}{2}}$ 25 Hz, 3α-H), and 5.32 (m, $w_{\frac{1}{2}}$ 12 Hz, 6-H) (Found: C, 70.9; H, 8.3. $C_{23}H_{32}O_5$ requires C, 71.7; H, 8.3%). Other major products were 3β-acetoxy-16α,17α-epoxypregn-5-en-20-one (**10**) (*k'* 0.44) and 3β,18-diacetoxy-16α,17α;18,20-diepoxypregn-5-ene (**17**) (*k'* 0.38) obtained as an apparently homogeneous gum; $\nu_{max.}$ 1 735, 1 250, and 1 030 cm^{-1}. The n.m.r. spectrum of the latter material showed it to be a mixture of four isomers (at C-18 and C-20), of which two were major components: δ 0.98 (s, 19-H_3), 1.20, 1.32 (centres of two doublets due to 21-H_3, *J* 7 Hz), 2.04 and 2.06 (2 s, 3 and 18-OAc), 3.41 and 3.51 (2 s, 16β-H), 4.2 (complex, 20-H), 4.54 (m, $w_{\frac{1}{2}}$ 26 Hz, 3α-H), 5.34 (m, $w_{\frac{1}{2}}$ 12 Hz, 6-H), and 5.88, 5.94, 6.22 and 6.30 (4 s, 18-H) (a detailed n.m.r. spectral analysis and individual configurational assignments for the four isomers will be published elsewhere; *m/z** 370 (M^+ − AcOH), 328 [M^+ − (AcOH + C_2H_2O)], 310 (M^+ − 2AcOH), 295 [M^+ − (2AcOH + CH_3)], 281 [M^+ − (AcOH − $C_3H_5O_3$)], 254 [M^+ − (AcOH + $C_5H_7O_3$)], 175 [M^+ − (2AcOH + CH_3 + C_9H_{12}†)],161 [M^+ − (AcOH + $C_3H_5O_3$ + C_9H_{12}†)].

A minor quantity of a product believed to be 3β,18-diacetoxy-16α,17α;18,20-diepoxypregn-5-en-7-one (**18**) (*k'* 2.88) was obtained as a gum, apparently homogeneous on h.p.l.c., $\nu_{max.}$ 1 735, 1 673, 1 633, 1 250, and 1 035 cm^{-1}; δ 1.18 (s, 19-H_3), 1.335 (d, *J* 7 Hz, 21-H_3), 2.04 (s, 3- and 18-OAc), 3.44 and 3.56 (2 s, 16β-H), 4.24 (complex, 20-H), 4.70 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.71 (br s, 6-H), and 6.24 and 6.34 (2 s, 18-H) (the doubling of signals of 16β-and 18-H indicated the presence of at least two isomers, at C-18 and/or C-20); *m/z*‡ 384 (M^+ − AcOH), 341 [M^+ − (AcOH + CH_3CO)], 324 (M^+ − 2AcOH), 309 [M^+ − (2AcOH + CH_3)], 295 [M^+ − (AcOH + $C_3H_5O_3$)], 268 [M^+ − (AcOH + $C_5H_7O_3$)], 268 [M^+ − (AcOH + $C_5H_7O_3$)], 253 [M^+ − (AcOH + $C_5H_7O_3$ + CH_3)], and 174 [M^+ − (AcOH + $C_{11}H_{14}O_4$)]. 16α,17α-Epoxy-18-iodopregn-5-en-20-one (**13**) (*k'* 0.33) was isolated from initial experiments when solvolysis was carried out for shorter periods, $\nu_{max.}$ 1 735, 1 705, 1 250, and 1 030 cm^{-1}; δ 1.06 (s, 19-H_3), 2.04 (s, 3-OAc), 2.13 (s, 21-H_3), 3.88 (s, 16β-H), 3.50 and 3.84 (dd, *J* 10 Hz, 18-H_2), 4.56 (m, $w_{\frac{1}{2}}$ 25 Hz, 3α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H). Because of its instability this compound was not characterised further.

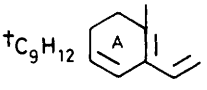
3β,18-*Diacetoxy*-16α,17α-*epoxypregn*-5-*en*-20-*one* (**15**).—3β-Acetoxy-16α,17α-epoxy-18-hydroxypregn-5-en-20-one (**14**) (464 mg) in pyridine (3 ml) was treated with acetic anhydride (0.46 ml) and left at room temperature overnight. Conventional work-up gave the crude *diacetate* (485 mg), m.p. (pure diacetate) 142—144 °C (from acetone–hexane); $\nu_{max.}$ 1 725—1 740, 1 695, 1 250, and 1 040 cm^{-1}; δ 1.07 (s, 19-H_3), 1.98 and 2.05 (2 s, 18- and 3-OAc), 2.10 (s, 21-H_3), 3.78 (s, 16β-H), 4.44 and 4.58 (dd, *J* 12 Hz, 18-H_2), 4.52 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H) (Found: C, 69.9; H, 7.9. $C_{25}H_{34}O_6$ requires C, 69.7; H, 8.0%).

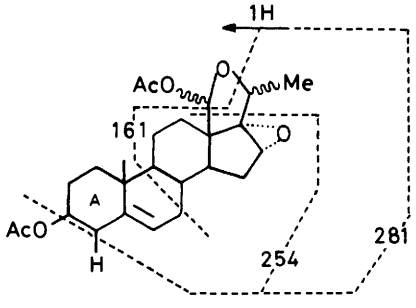
Preparation of 3β,16α,18-*Trihydroxyandrost*-5-*en*-17-*one* (**1**)

3β,18-*Diacetoxy*-16α,17α-*epoxypregn*-5-*en*-20-*one Oxime* (**16**).—Hydroxylamine hydrochloride (84.0 mg, 1.209 mmol) was added to a solution of the ketone (**15**) (100 mg, 0.232 mmol) in pyridine (2 ml) which was left for 3 days at room temperature. The solution was diluted with water and the product was extracted with ethyl acetate; the extract was then washed with 3M-hydrochloric acid and water until neutral. The solvent was dried ($MgSO_4$) and removed under reduced pressure to give the *oxime* (**16**) (90 mg), m.p. 168—171 °C (from acetone–hexane); $\nu_{max.}$ 3 430, 1 730, 1 720sh, 1 250, and 1 040 cm^{-1}; δ 1.03 (s, 19-H_3), 1.92 (s, 21-H_3), 2.02 and 2.04 (2 s, 3- and 18-OAc), 3.66 (s, 16β-H), 4.14 and 4.30 (dd, *J* 12 Hz, 18-H_2), 4.54 (m, $w_{\frac{1}{2}}$ 20 Hz, 3α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H) (Found: C, 67.3; H, 7.9; N, 3.1. $C_{25}H_{35}NO_6$ requires C, 67.3; H, 7.9; N, 3.4%).

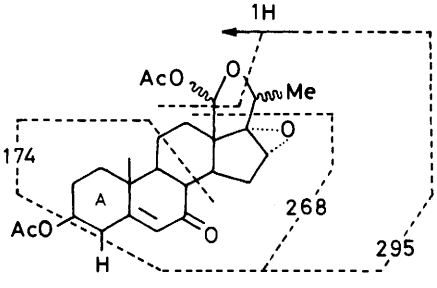
Beckmann Rearrangement of the 20-*Oxime* (**16**).—A solution of the oxime (**16**) (45 mg, 0.10 mmol) in dry pyridine (1.31 ml) was cooled to −15 °C and then stirred during the addition of phosphoryl chloride in pyridine [0.72 ml of a solution of $POCl_3$ (0.9 ml) in pyridine (1.5 ml)]; the temperature of the mixture was maintained below −10 °C during the addition and for a further 1 h, before being allowed to rise to 0 °C for 3 h. Ice was then added to the red solution, which was then concentrated under reduced pressure and extracted with ethyl acetate. The extract was washed with 3M-hydrochloric acid, aqueous sodium hydrogen carbonate, and water until neutral, after which the

* Tentative fragmentation pattern of compound (**17**).





‡ Tentative fragmentation pattern of compound (**18**).



solvent was removed under reduced pressure to give 3β,16α,18-*triacetoxyandrost-5-en*-17-*one* (**19**) which crystallised (15.1 mg) from aqueous methanol as needles, m.p. 129—131 °C; $\nu_{max.}$ 1 745, 1 735, and 1 250 cm^{-1}; δ 1.06 (s, 19-H_3), 2.14 and 2.04 (2 s, 3β-, 16α-, and 18-OAc), 4.25 and 4.35 (dd, *J* 12 Hz, 18-H_2), 4.62 (m, $w_{\frac{1}{2}}$ 28 Hz, 3α-H), and 5.46 (overlapping multiplets, $w_{\frac{1}{2}}$ 22 Hz, 16β- and 6-H); *m/z* 386 (M^+ − AcOH), 313 [M^+ − (AcOH + $CH_3CO_2CH_2$)], 266 [M^+ − (AcOH + C_9H_{12}*)], and 253 [M^+ − (2AcOH + $CH_3CO_2CH_2$)] (Found: C, 66.9; H, 7.7. $C_{25}H_{34}O_7$ requires C, 67.2; H, 7.7%). A small amount of 3β,18-*diacetoxy*-16α-*hydroxyandrost*-5-*en*-17-*one* (**20**) was also isolated, $\nu_{max.}$ 3 500, 1 745, 1 730, and 1 245 cm^{-1}; δ 1.04 (s, 19-H_3), 2.04 (s, 3- and 18-OAc), 4.24 and 4.39 (dd, *J* 12 Hz, 18-H_2), 4.40—4.80 (m, 3α- and 16β-H), and 5.38 (m, $w_{\frac{1}{2}}$ 11 Hz, 6-H) (Found: C, 68.1; H, 7.7. $C_{23}H_{32}O_6$ requires C, 68.3; H, 7.9%).

3β,16α,18-*Triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*one Diphenylhydrazone* (**23**).—1,1-Diphenylhydrazine (0.03 g, 0.209 mmol) was added to a solution of 3β,18-diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) (50 mg, 0.116 mmol) in acetic acid (0.4 ml) and allowed to stand for 3 days. The dark mixture was poured into water and the product was extracted with ethyl acetate. The organic phase was washed with aqueous sodium hydrogen carbonate and water and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a brown gum from which the required diphenylhydrazone (**23**) was isolated with difficulty by preparative t.l.c. or h.p.l.c. as an apparently homogeneous but unstable pale yellow solid (69.6 mg), $\nu_{max.}$ 3 450, 1 735, 1 590, 1 245, 1 040, 760, and 710 cm^{-1}; δ 1.03 (s, 19-H_3), 1.72 (s, 16-OAc), 1.86 (s, 18-OAc), 2.04 (s, 3-OAc), 2.12 (s, 21-H_3), 3.36 (s, 17-OH), 3.87 and 4.35 (dd, *J* 12 Hz, 18-H_2), 4.58 (m, $w_{\frac{1}{2}}$ 26 Hz, 3α-H), 5.38 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), 6.22 (m, $w_{\frac{1}{2}}$ 16 Hz, 16β-H), and 6.90—7.38 (aromatic protons).

3β,16α,18-*Triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*one* (**25**).—(*a*) *With pyruvic acid.* The 20-diphenylhydrazone (**23**) (37 mg, 0.056 mmol) in acetic acid (0.16 ml) was treated with aqueous pyruvic acid (0.02 ml; 50% v/v) for 1 h at 100 °C after which water (0.15 ml) was added and the mixture held at the same temperature for another 15 min before being cooled. The mixture was diluted with water and the product extracted with ethyl acetate; the extract was washed with saturated aqueous hydrogen carbonate and water and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 3β,16α,18-*triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*one* (**35**) (14.4 mg) (see below) and 16α,18-diacetoxy-3β,17α-dihydroxypregn-5-en-20-one (2 mg) (separated by h.p.l.c.), $\nu_{max.}$ 3 450, 1 730, 1 240, and 1 040 cm^{-1}; δ 1.00 (s, 19-H_3), 2.00 and 2.06 (16-and 18-OAc), 2.30 (s, 21-H_3), 3.17 (s, 17-OH), 3.52 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 3.79 and 4.21 (dd, *J* 12 Hz, 18-H_2), 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 5.86 (m, $w_{\frac{1}{2}}$ 16 Hz, 16α-H); *m/z* (FAB) 449 (M + H)$^+$, 431 [(M + H)$^+$ − H_2O], 389 [(M + H)$^+$ − AcOH], 329 [(M + H)$^+$ − 2AcOH], and 311 [(M + H)$^+$ − (H_2O + C_9H_{12}*)].

(*b*) *With* p-*hydroxybenzaldehyde.* To a solution of the 20-diphenylhydrazone (**23**) (1.55 g, 2.363 mmol) in acetic acid (46 ml) and water (23 ml), *p*-hydroxybenzaldehyde (4.329 g, 35.44 mmol) and sodium acetate (332.9 mg) were added. The reaction mixture was left at 60 °C under nitrogen for 24 h, after which it was poured into ice–water and extracted with ethyl acetate. The organic layer was washed with 5% aqueous sodium hydroxide until there was no red colour in the aqueous layer after which it was dried ($MgSO_4$); the solvent was then removed to give a brown gum. Preparative h.p.l.c. afforded 3β,16α,18-triacetoxy-17α-hydroxypregn-5-en-20-one (**25**) (918.8 mg), and a small quantity of 16α,18-diacetoxy-3β,17α-dihydroxypregn-5-en-20-one. The triacetate (**25**) crystallised from acetone–hexane as needles, m.p. 210—211 °C; $\nu_{max.}$ 3 450, 1 730, 1 700sh, 1 245, and 1 040 cm^{-1}; δ 1.00 (s, 19-H_3), 2.00, 2.03, and 2.06 (3 s, 3-, 16-, and 18-OAc), 2.29 (s, 21-H_3), 3.17 (s, 17-OH), 3.79 and 4.21 (dd, *J* 12 Hz, 18-H_2), 4.52 (m, $w_{\frac{1}{2}}$ 28 Hz, 3α-H), 5.34 (m, $w_{\frac{1}{2}}$ 11 Hz, 6-H), and 5.86 (m, $w_{\frac{1}{2}}$ 16 Hz, 16β-H) (Found: C, 66.5; H, 7.8. $C_{27}H_{38}O_8$ requires C, 66.1; H, 7.8%).

3β,16α,18-*Triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*one Ethoxycarbonylhydrazone* (**24**).—Ethoxycarbonylhydrazine (7.2 mg, 0.07 mmol) was added to 3β,18-diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) (10 mg, 0.023 mmol) in acetic acid (0.17 ml) and the mixture was stirred for 48 h; it was then poured into ice–water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and water until neutral. The extract was dried ($MgSO_4$) and the solvent removed to give a crystalline solid. H.p.l.c. gave the 20-*ethoxycarbonylhydrazone* (**24**) (8 mg), m.p. 236—238 °C (needles from acetone–hexane); $\nu_{max.}$ 3 450, 3 360, 1 730, 1 710sh, 1 250, and 1 040 cm^{-1}; δ 1.00 (s, 19-H_3), 1.29 (t, *J* 7 Hz, CH_3CH_2), 1.86 (s, 16-OAc), 1.98 (s, 18-OAc), 2.02 (s, OAc), 2.06 (s, 21-H_3), 2.94 (s, 17-OH), 3.75 and 4.25 (dd, *J* 12 Hz, 18-H_2), 4.22 (q, *J* 6 Hz, CH_2CH_3), 4.56 (m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), 6.12 (m, $w_{\frac{1}{2}}$ 18 Hz, 16β-H), and 7.46 (s, NH) (Found: C, 62.0; H, 7.7; N, 4.8. $C_{30}H_{44}N_2O_9$ requires C, 62.4; H, 7.7; N, 4.9%).

Regeneration of C-20 *Ketone from the* 20-*Ethoxycarbonylhydrazone* (**24**).—The 20-ketone (**25**) was regnerated from the 20-ethoxycarbonylhydrazone (**24**) with *p*-hydroxybenzaldehyde under the same conditions as used above for the 20-diphenylhydrazone (**23**). The yield (65%) of the regenerated ketone from (**24**) was slightly lower than from (**23**), but the product was much cleaner.

3β,16α,18-*Triacetoxypregn*-5-*ene*-17α,20β-*diol* (**26**).—The 20-ketone (**25**) (24 mg, 0.49 mmol) in dry tetrahydrofuran (4.2 ml) and dry methanol (2.1 ml) was cooled in ice and then sodium borohydride (37.0 mg, 0.97 mmol) was added portionwise. Reduction was complete after 1 h. Acetic acid (0.5 ml) was then added and the steroid was precipitated by removing most of the solvent and adding water. The dried product crystallised from acetone–hexane to give the 17α,20β-*diol* (**26**) as needles, m.p. 189—191 °C; $\nu_{max.}$ 3 520, 1 730, 1 250, and 1 040 cm^{-1}; δ 1.04 (s, 19-H_3), 1.08 (d, *J* 8 Hz, 21-H_3), 2.04 (s, 3-OAc), 2.12 (s, 16α- and 18-OAc), 2.66 (s, 17-OH), 3.86 (m, $w_{\frac{1}{2}}$ 24 Hz, 20α-H), 4.12 and 4.32 (dd, *J* 12 Hz, 18-H_2), 4.60 (m, *J* 24 Hz, 3α-H), 5.02 (m, $w_{\frac{1}{2}}$ 24 Hz, 16β-H), and 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H); δ(C_5D_5N), 1.02 (s, 19-H_3), 1.54 (d, *J* 6 Hz, 21-H_3), 2.08, 2.09, and 2.16 (3 s, 3-, 16-, and 18-OAc), 4.36 (q, *J* 6 Hz, 20-H), 4.68 (m, 3α-H, and s, 18-H_2), 5.30 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 5.60 (m, $w_{\frac{1}{2}}$ 16 Hz, 16β-H) (Found: C, 65.9; H, 8.2. $C_{27}H_{40}O_8$ requires C, 65.9; H, 8.1%).

Cleavage of the Pregnane Side Chain to give 3β,16α,18-*Triacetoxyandrost*-5-*en*-17-*one* (**19**).—(*a*) *With sodium metaperiodate.* Sodium metaperiodate (223.9 mg, 1.047 mmol) and water (0.56 ml) were added to a solution of 3β,16α,18-triacetoxypregn-5-ene-17α,20β-diol (**26**) (103 mg, 0.209 mmol) in methanol (6 ml) which was stirred at room temperature for 3 days. Water (5 ml) was then added to dissolve the precipitated salts and the product was extracted with ethyl acetate, which was washed with water and dried ($MgSO_4$). Evaporation of the solvent left a gum which afforded (h.p.l.c.) the 3,16,18-triacetate (**19**) (54.1 mg) (see above) and a minor quantity (5.9 mg) of an unidentified compound, $\nu_{max.}$ 3 510, 1 730, 1 245, 1 100, and 1 035 cm^{-1}; δ 1.04 (s, 19-H_3), 1.17 (d, *J* 7 Hz, 21-H_3), 2.04 and 2.14 (2 s, -OAc), 4.29 (q, *J* 6 Hz), 4.59 (m, $w_{\frac{1}{2}}$ 30 Hz), 4.56 (s), 5.34 (m, $w_{\frac{1}{2}}$ 12 Hz, 6-H), and 5.54 (br s).

† See footnote * p. 1827.

(*b*) *With lead tetra-acetate.* The diol (**26**) (500 mg, 1.016 mmol) in acetic acid (5 ml) was treated with lead tetra-acetate (540.2 mg, 1.22 mmol) with stirring for 15 h after which ice–water was added to destroy excess of the reagent. After filtration through Celite, the filtrate was extracted with ethyl acetate; the extract was washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and evaporated to furnish the triacetate (**19**) (432 mg), identical with the material described above.

3β,16α,18-*Trihydroxyandrost*-5-*en*-17-*one* (**1**).—(*a*) *Chemical method.* The triacetate (**19**) (260 mg, 0.583 mmol) in methanolic hydrochloric acid (40.9 ml; methanol:HCl:water 86:4:10) was allowed to stand for 24 h at 45 °C. Water was then added to the mixture and the methanol evaporated under reduced pressure. The precipitated and dried product was essentially a single compound (h.p.l.c.) which crystallised from methanol to give 3β,16α,18-*trihydroxyandrost*-5-*en*-17-*one* (**1**), m.p. 217—219 °C; $\nu_{max.}$ 3 350 and 1 745 cm^{-1}, δ(C_5D_5N) 1.00 (s, 19-H_3), 3.78 (m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 4.20 (d, *J* 6 Hz, 18-H_2), 4.88 (d, *J* 7 Hz, 16β-H), 5.40 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), 6.18 (d, *J* 4 Hz, 3-OH), 6.54 (t, *J* 6 Hz, 18-OH), and 7.34 (d, *J* 4 Hz, 16-OH) (Found: C, 71.2; H, 8.8. $C_{19}H_{28}O_4$ requires C, 71.2; H, 8.8%). Mass spectrum of 17-methoxyimino-3,16α,18-tris(trimethylsilyl) (MO-TMS) derivative, *m/z* 565 (M^+), 534 (M^+ − OMe), and 444 [M^+ − (TMSOH + OMe)].

(*b*) *Enzymic hydrolysis.* The triacetate (**19**) (2 mg, 0.004 mmol) was dissolved in ethanol (1 ml) by heating; the mixture was then cooled to room temperature and added immediately to sodium acetate buffer[38] (20 ml: 0.2M; pH 4.6) containing the *Helix pomatia* enzyme (0.2 ml). After 2.5 days at 36 °C the dark brown colloidal solution was centrifuged for 20 min and the deep yellow supernatant aqueous solution was extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and taken to dryness to give a pale yellow solid (1.8 mg). T.l.c. showed this material to be the required 3β,16α,18-triol (**1**), apart from pigments which remained at the baseline. G.c.–mass spectral analysis (as MO-TMS derivative) confirmed the identity of the products.

Androst-5-*ene*-3β,16α,17β,18-*tetraol* (**34**).—Sodium borohydride (18.2 mg, 0.48 mmol) was added portionwise to a stirred solution of 3β,16α,18-trihydroxyandrost-5-en-17-one (**1**) (76.8 mg, 0.24 mmol) in dry methanol (6 ml) and dry tetrahydrofuran (16.5 ml) at 0 °C. After the mixture had been stirred for a further 2 h the reaction was stopped by destroying excess of sodium borohydride with acetic acid. The steroid (66.7 mg) was then precipitated with water after evaporation of most of the organic solvent under reduced pressure. H.p.l.c. on a reverse-phase column showed it to be a single compound (*k'* 1.56), which on crystallisation from methanol gave the 3β,16α,17β,18-*tetraol*, m.p. 230—233 °C, $\nu_{max.}$ 3 400 and 1 055 cm^{-1}; δ(C_5D_5N) 1.02 (s, 19-H_3), 3.80 (m, $w_{\frac{1}{2}}$ 18 Hz, 3α-H), 4.14 (br s, 18-H_2), 4.26 (m, d on adding D_2O, *J* 6 Hz, 17α-H), 4.88 (m, $w_{\frac{1}{2}}$ 14 Hz, 16β-H), 5.26 (d, *J* 5 Hz, 17-OH), 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H and 18-OH), 6.16 (d, *J* 5 Hz, 3-OH), and 6.62 (d, *J* 5 Hz, 16-OH) (Found: C, 68.9; H, 9.4. $C_{19}H_{38}O_4 \cdot 0.5H_2O$ requires C, 68.8; H, 9.4%). Mass spectrum of tetrakis(trimethylsilyl) (TMS) derivative, *m/z* 595 (M^+ − CH_3), 520 (M^+ − TMSOH), 505 [M^+ − (TMSOH + CH_3)], 430 [M^+ − (2 × TMSOH)], 417 [M^+ − (TMSOH + CH_2OTMS)], 327 [M^+ − (2 × TMSOH + CH_2OTMS)], and 237 [M^+ − (3 × TMSOH + CH_2OTMS)].

17β,18-*Isopropylidenedioxyandrost*-5-*ene*-3β,16α-*diol* (**26**) *and Its Diacetate* (**37**).—The 3β,16α,17β,18-tetraol (**34**) (11.3 mg, 0.035 mmol) was treated with 2,2-dimethoxypropane (0.36 ml) in dimethylformamide (0.06 ml) containing toluene-*p*-sulphonic acid (0.4 mg) under reflux for 3 h. The solvents were removed under reduced pressure and the residue was extracted with ethyl acetate; the extract was washed with aqueous sodium hydrogen carbonate and water until neutral, dried (Na_2CO_3), and evaporated under reduced pressure to give the acetonide (**36**) (7.3 mg), m.p. 233—235 °C (needles from acetone–hexane); $\nu_{max.}$ 3 240, 3 260, 1 370, 1 240, and 1 055 cm^{-1}; δ(C_5D_5N) 1.02 (s, 19-H_3), 1.50 (s, acetonide methyls), 3.77 (br s, 18-H_2), 3.80 (m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 4.16 (m, $w_{\frac{1}{2}}$ 4 Hz, 17α-H), 4.82 (m, $w_{\frac{1}{2}}$ 9 Hz, 16β-H), 5.38, (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), 6.18 (d, *J* 6 Hz, 3-OH), and 6.58 (d, *J* 4 Hz, 16-OH) (Found: C, 72.7; H, 9.3. $C_{22}H_{34}O_4$ requires C, 72.8; H, 9.4%).

The foregoing compound (**36**) was acetylated (acetic anhydride–pyridine) at room temperature to give the 3,16-diacetate (**37**) as a gum, homogeneous on h.p.l.c., $\nu_{max.}$ 1 730, 1 250, and 1 045 cm^{-1}; δ(C_5D_5N) 0.93 (s, 19-H_3), 1.48 and 1.56 (2 s, acetonide methyls), 2.06 (s, 3- and 18-OAc), 3.74 (br s, 18-H_2), 4.03 (d, *J* 3 Hz, 17α-H), 4.58 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.30 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 5.70 (m, $w_{\frac{1}{2}}$ 12 Hz, 16β-H); *m/z* (FAB) 447 (M + H)$^+$.

Preparation of 3β,16β,18-*Triacetoxyandrost*-5-*en*-17-*one* (**22**)

3β,16β,18-*Triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*one* (**27**).—3α,18-Diacetoxy-16α,17α-epoxypregn-5-en-20-one (**15**) (100 mg, 0.233 mmol) in acetic acid containing 10% sulphuric acid (1.8 ml) was stirred at room temperature. T.l.c. showed gradual formation of the triacetate (**27**). After 6 h a further transformation product (**30**) of intermediate polarity began to appear. Ice was then added to the mixture which was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and taken to dryness under reduced pressure. The 3β,16β,18-*triacetate* (**27**) (58 mg) was purified by h.p.l.c. (30% ethyl acetate in hexane) (*k'* 3.58), m.p. 164—166 °C from acetone–hexane; $\nu_{max.}$ 3 460—3 420, 1 735, 1 715, 1 250, and 1 045 cm^{-1}; δ 1.02 (s, 19-H_3), 1.98 (s, 21-H_3), 2.04 and 2.06 (2 s, 3- and 18-OAc), 3.56 (s, 17-OH), 4.12 and 4.28 (dd, *J* 12 Hz, 18-H_2), 4.62 (m, $w_{\frac{1}{2}}$ 20 Hz, 3α- and 16α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H) (Found: C, 65.8; H, 7.8. $C_{27}H_{38}O_8$ requires C, 66.1; H, 7.8%).

The product of further reaction was probably 3β,16β,diacetoxy-17aβ,18-dihydroxy-17aα-methyl-D-homoandrost-5-en-17-one (**30**) obtained as an amorphous solid (single peak on h.p.l.c., *k'* 1.06) (5 mg), m.p. (crude) 179—186 °C; $\nu_{max.}$ 3 420, 1 760, 1 735, 1 725sh, 1 260sh, 1 240, and 1 040 cm^{-1}; δ 0.94 (s, 19-H_3), 1.21 (s, 17aα-CH_3), 2.02 and 2.1 (2 s, OAc), 3.80 and 4.36 (dd, *J* 8 Hz, 18-H_2), 4.66 (m, $w_{\frac{1}{2}}$ 28 Hz, 16α-H), and 5.32 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H). The 17aβ-hydroxy-17aα-methyl configuration was assigned on the basis of nuclear Overhauser difference experiments[39] at 400 MHz in which irradiation of the 17a-methyl proton signal at δ 1.21 caused enhancement of the 16α-H signal at δ 4.66 but no enhancement of either of the signals due to protons at C-18. Similarly, separate irradiation of each proton at C-18 showed no evidence of proximity to the 17a-methyl substituent. That the by-product (**30**) was the 17-one rather than a 17a-one was deduced from the i.r. band at 1 760 cm^{-1} which was characteristic of an α-acetoxy keto-grouping. Mass spectrum, *m/z* 404 (M^+ − C_2H_4O), 388 (M^+ − AcOH), 370 [M^+ − (AcOH + H_2O)], 328 (M^+ − 2AcOH), and 310 [M^+ − (2AcOH + H_2O)].

3β,16β,18-*Triacetoxy*-17α-*hydroxypregn*-5-*en*-20-*ols* (**28**).—The 20-ketone (**27**) (81.7 mg, 0.167 mmol) in dry methanol (0.7 ml) and dry tetrahydrofuran (1.4 ml) at 0 °C was treated with sodium borohydride (12.6 mg, 0.333 mmol). The mixture was

stirred at 0 °C for 3 h after which excess of the reagent was destroyed by adding acetic acid and the product was isolated in the usual way as a gum (89 mg) which comprised mainly the required 20β- and 20α-alcohols. The mixture exhibited $\nu_{max.}$ 3 480—3 440, 1 730, 1 249, and 1 040 cm^{-1}; h.p.l.c. separation (40% EtOAc in hexane) of small samples of the two isomers (20β and 20α) showed that they were present approximately in the ratio 1:1. They were isolated as gums, each apparently homogeneous (h.p.l.c.), with the following characteristics. 20β-*Alcohol*: δ 1.04 (s, 19-H_3), 1.14 (d, *J* 8 Hz, 21-H_3), 2.01, 2.03, and 2.15 (3 s, 3-, 16-, and 18(OAc), 4.11 and 4.53 (dd, *J* 12 Hz, 18-H_2), 4.20 (m, $w_{\frac{1}{2}}$ 24 Hz, 20α-H), 4.54 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 4.82 (m, $w_{\frac{1}{2}}$ 18 Hz, 16α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H); δ(C_5D_5N), 1.04 (s, 19-H_3), 1.51 (d, *J* 6 Hz, 21-H_3), 2.04, 2.10, and 2.16 (3 s, 3-, 16-, and 18-OAc), 4.50—5.10 (3α-H, 18-H_2, and 20α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 12 Hz, 6-H and 16α-H). 20α-*Alcohol*: δ 1.02 (s, 19-H_3), 1.30 (d, *J* 8 Hz, 21-H_3), 2.04, 2.08, and 2.09 (3 s, 3-, 16-, and 18-OAc), 3.94 (m, $w_{\frac{1}{2}}$ 27 Hz, 20β-H), 4.23 and 4.33 (dd, *J* 12 Hz, 18-H_2), 4.50 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 4.92 (m, $w_{\frac{1}{2}}$ 16 Hz, 16α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H); δ(C_5D_5N) 1.04 (s, 19-H_3), 1.70 (d, *J* 8 Hz, 21-H_3), 2.04, 2.08, and 2.12 (3 s, 3-, 16-, and 18-OAc), 4.64 (m, $w_{\frac{1}{2}}$ 27 Hz, 3α-H and 18-H_2), 4.90 (m, $w_{\frac{1}{2}}$ 24 Hz, 20β-H), 5.30 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H), and 5.66 (m, $w_{\frac{1}{2}}$ 12 Hz, 16α-H). Mass spectrum of unseparated 20-alcohols (**28**), *m/z* (FAB) 493 $(M + H)^+$, 475 $[(M + H)^+ - H_2O]$, 433 $[(M + H)^+ - AcOH]$, 415 $[(M + H)^+ (H_2O + AcOH)]$, 313 $[(M + H)^+ - (AcOH + C_9H_{12}*)]$, and 295 $[(M + H)^+ - (H_2O + 2AcOH)]$.

A small amount of a crude product believed to be the 3,18,20-*triacetate* (**29**) was also obtained (h.p.l.c., single peak), evidently as a product of acetyl migration from the 16β- to the 20-hydroxy position; δ 1.03 (s, 19-H_3), 1.40 (d, *J* 6 Hz, 21-H_3), 4.12—4.64 (overlapping dd, 18-H_2 and m, 3α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 14 Hz, 20-H and 6-H). The location of the third acetate at C-20 was confirmed by a double irradiation experiment on the signal due to the C-20 proton, which was shifted by acetylation to δ 5.36: the 21-methyl doublet collapsed to a singlet. Mass spectrum, *m/z* (FAB) 493 $(M + H)^+$, 475 $[(M + H)^+ - H_2O]$, 433 $[(M + H)^+ - AcOH]$, 415 $[(M + H)^+ - (H_2O + AcOH)]$, 373 $[(M + H)^+ - 2AcOH]$, 355 $[(M + H)^+ - (H_2O + 2AcOH)]$, 313 $[(M + H)^+ - (AcOH + C_9H_{12}*)]$, and 295 $[(M + H)^+ - (H_2O + C_9H_{12}*)]$.

3β,16β,18-*Triacetoxyandrost-5-en-17-one* (**22**).—(*a*) *With sodium metaperiodate.* The unseparated 17α,20-diols (**28**) (15 mg, 0.030 mmol) in methanol (1.15 ml) and water (0.08 ml) were treated with sodium metaperiodate (9.8 mg, 0.046 mmol). Reaction was very slow, so a further quantity of periodate (9.8 mg) was added on the second day. After 6 days, when the reaction was still incomplete, the product was isolated as in the 16α-sequence above. Preparative t.l.c. furnished 3β,16β,18-triacetoxyandrost-5-en-17-one (**22**) (8.4 mg) (see below).

(*b*) *With lead tetra-acetate.* Lead tetra-acetate (32 mg, 0.072 mmol) was added to a solution of the 17α,20-diol (**28**) (30 mg, 0.061 mmol) in dry glacial acetic acid (0.3 ml). The mixture was stirred at room temperature for 20 h and then worked up as previously to give the 3β,16β,18-triacetate (**22**) (14 mg), m.p. 168—169 °C (needles from acetone–hexane); $\nu_{max.}$ 1 760sh, 1 740, 1 255, and 1 230 cm^{-1}; δ 1.06 (s, 19-H_3), 2.04, 2.08, and 2.13 (3 s, 3-, 16-, and 18-OAc), 3.11 (br s, 18-H_2), 4.57 (m, $w_{\frac{1}{2}}$ 30 Hz, 3α-H), 5.02 (t, *J* 8 Hz, 16α-H), and 5.39 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H); *m/z* 446 (M^+), 386 $(M^+ - AcOH)$, 313 $[M^+ - (AcOH + CH_2CO_2CH_3)]$, 266 $[M^+ - (AcOH + C_9H_{12}*)]$, and 253 $[M^+ - (2AcOH + CH_2CO_2CH_3)]$ (Found: C, 67.5; H, 7.4. $C_{25}H_{34}O_7$ requires C, 67.2; H, 7.7%).

Attempted Hydrolysis of 3β,16β,18-*Triacetoxyandrost-5-en-17-one* (**22**) *with* Helix pomatia.—The *Helix pomatia* enzyme (0.53 ml) was added to a solution of the 16β-triacetate (**22**) (5 mg, 0.011 mmol) in ethanol (2.3 ml) diluted with sodium acetate buffer (45 ml; 0.2M; pH 4.65). The brown solution was then left at 36 °C for 2.5 days. After cooling the dark brown colloidal solution was centrifuged for 15 min and the deep yellow supernatant aqueous solution was then extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and taken to dryness to give a gum. H.p.l.c. separation on a reverse phase column gave the isomerised 17-hydroxy-16-ketone (**31**) (3.5 mg) as a solid, m.p. (crude) 165—170 °C; $\nu_{max.}$ 3 430, 1 745, and 1 055 cm^{-1}; δ(C_5D_5N) 1.04 (s, 19-H_3), 3.84 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 4.10 (br s, 17α-H), 4.18 (s, 18-H_2), 5.38 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H), 5.64 (t, *J* 6 Hz, 18-OH), and 6.23 (3 and 17-OH). Mass spectrum of 16-methoxyimino-3β,17β,18-tris(trimethylsilyl) (MO-TMS) derivative, *m/z* 565 (M^+), 534 $(M^+ - OMe)$, 475 $(M^+ - TMSOH)$, 444 $[M^+ - (TMSOH + OMe)]$, 417 $[M^+ - (TMSOH + CH_2OTMS)]$, 391 $[M^+ - (CH_2OTMS + CH_2CNOMe)]$, 354 $[M^+ - (OMe + 2TMSOH)]$, 282 $[M^+ - (CH_2OTMS + 2TMSOH)]$, 211 $[M^+ - (CH_2OTMS + 2TMSOH + CH_2CNOMe)]$.

Acetylation (Ac_2O–pyridine) gave the 3β,17β,18-triacetate (**32**) as a homogeneous gum (t.l.c.), $\nu_{max.}$ 1 740—1 720, 1 250, and 1 050 cm^{-1}; δ 1.06 (s, 19-H_3), 1.96 (s, 17-OAc), 2.04 (s, 3-OAc), 2.15 (s, 18-OAc), 4.18 and 4.36 (dd, *J* 12 Hz, 18-H_2), 4.58 (m, $w_{\frac{1}{2}}$ 24 Hz, 3α-H), 5.04 (s, 17α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H).

Pregn-5-ene-3β,16β,17α,18,20-*pentaols* (**33**).—A solution of 3β,16β,18-triacetoxy-17α,20-dihydroxypregn-5-ene (**28**) (20 mg, 0.041 mmol) in methanol (3.6 ml) was treated with 2% aqueous sodium hydrogen carbonate (0.63 ml) and heated under reflux for 2 h under nitrogen. The solution was then concentrated under reduced pressure and extracted with ethyl acetate; the extract was washed with water, dried ($MgSO_4$), and evaporated to give a white solid (**33**) (10.8 mg). H.p.l.c. methanol–water 4:6 on a reverse phase column separated the 20α-and 20β-isomers (ratio 3:2 approximately), as amorphous solids, each giving a single peak, with the following characteristics. 20β-*Pentaol*: *k'* 3.9; $\nu_{max.}$ 3 400 and 1 045 cm^{-1}; δ(C_5D_5N), 1.09 (s, 19-H_3), 1.89 (d, *J* 7 Hz, 21-H_3), 3.78 (m, $w_{\frac{1}{2}}$ 20 Hz, 3α-H), 4.26 and 4.60 (dd, *J* 11 Hz, 18-CH_2), 4.61 (m, $w_{\frac{1}{2}}$ 20 Hz, 16α-H), 5.09 (s, 17-OH), 5.23 (q, *J* 7 Hz, 20-H), 5.38 (m, 6-H and OH), 6.12 (d, *J* 5 Hz, 3-OH), 6.54 (d, *J* 5 Hz, 20-OH), and 6.78 (t, *J* 4 Hz, 18-OH). 20α-*Pentaol*: *k'* 4.8; $\nu_{max.}$ 3 420 and 1 050 cm^{-1}; δ(C_5D_5N), 1.02 (s, 19-H_3), 1.89 (d, *J* 7 Hz, 21-H_3), 3.78 (m, $w_{\frac{1}{2}}$ 20 Hz, 3α-H), 4.13 and 4.55 (dd, *J* 11 Hz, 18-H_2), 4.71 (m, $w_{\frac{1}{2}}$ 26 Hz, 16α-H), 5.03 (s, 17-OH), 5.15 (q, *J* 7 Hz, 20-H) 5.38 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H), 5.80 (br s, 16-OH), 5.94 (d, *J* 4 Hz, 20-OH), and 6.16 (m, $w_{\frac{1}{2}}$ 12 Hz, 3- and 18-OH). The isomers gave virtually the same mass spectrum, *m/z* (FAB) 367 $(M + H)^+$, 349 $[(M + H)^+ - H_2O]$, 313 $[(M + H)^+ - 2H_2O]$, 313 $[(M + H)^+ - 3H_2O]$, and 211 $[(M + H)^+ - (2H_2O + C_9H_{12}*)]$.

Attempted Selective Periodate Cleavage of Pregn-5-ene-3β,16β,17α,18,20-*pentaols* (**33**).—The unseparated pentaols (**33**) (10.8 mg, 0.03 mmol) in methanol (1.8 ml) were treated with sodium metaperiodate solution (0.148 ml of a solution containing 1.0695 g in 25 ml of water). The mixture was stirred in the dark at room temperature for 19 h. Working up the product in the usual way led only to recovery of the pentaol (**33**).

Androst-5-ene-3β,16β,17β,18-*tetraol* (**35**).—A solution of 3β,16β,18-triacetoxyandrost-5-en-17-one (**22**) (73.6 mg, 0.165 mmol) in dry methanol (1.4 ml) and dry tetrahydrofuran (7.3 ml) at 0 °C was stirred with sodium borohydride (12.5 mg, 0.33 mmol) for 2 h, when reduction was complete (t.l.c.). The excess

* See footnote † p. 1827.

of borohydride was destroyed with acetic acid, and after evaporation of the solvents the product was extracted with ethyl acetate which was washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated under reduced pressure. The gummy product was mainly the tetraol 3,16,18-triacetate, with a small proportion of the 3,16-diacetate (n.m.r.). Deacetylation was completed by reaction with aqueous 2% sodium hydrogen carbonate (2.57 ml) under reflux in nitrogen for 2 h. Extraction by use of ethyl acetate gave the 3β,16β,17β,18-*tetraol* (**35**), m.p. 231—233 °C (needles from ethyl acetate–methanol); $\nu_{max.}$ 3 410, 1 075, and 1 045 cm^{-1}; δ(C_5D_5N) 1.02 (s, 19-H_3), 3.78 (d, *J* 6 Hz, 17α-H), 3.80 (m, $w_{\frac{1}{2}}$ 20 Hz, 3α-H), 4.09 and 4.53 (dd, *J* 12 Hz, 18-H_2), 4.46 (m, $w_{\frac{1}{2}}$ 20 Hz, 16α-H), 5.38 (m, *J* 10 Hz, 6-H), 5.66, 6.04, 6.16, and 6.44 (each br s, removed by D_2O, O*H*); mass spectrum [as tetrakis(trimethylsilyl) (TMS) derivatives], *m/z* 595 ($M^+ - CH_3$), 520 (M^+ − TMSOH), 430 [M^+ − (2TMSOH)], 417 [M^+ − (TMSOH + CH_2OTMS)], 327 [M^+ − (2TMSOH + CH_2OTMS)], 237 [M^+ − (3TMSOH + CH_2OTMS)], and 207 [M^+ − (3TMSOH + CH_2OTMS + C_9H_{12}*)] (Found: C, 70.4; H, 9.4. $C_{19}H_{30}O_4$ requires C, 70.7; H, 9.4%).

16β,17β-*Isopropylidenedioxyandrost-5-ene*-3β,18-*diol* (**38**) *and Its Diacetate* (**39**).—The isopropylidenedioxy derivative (**38**) was prepared from the tetraol (**35**) as for the 16α-epimer (**34**), although the reaction was faster. It was obtained as a crude solid (one spot on t.l.c.), $\nu_{max.}$ 3 500, 3 420, 1 050, and 870 cm^{-1}; δ 1.04 (s, 19-H_3), 1.34 and 1.58 (2 s, acetonide methyls), 3.30—3.92 (m, 3α-H and overlapping dd, 18-H_2), 4.10 (d, *J* 8 Hz, 17α-H), 4.64 (m, $w_{\frac{1}{2}}$ 20 Hz, 16α-H), and 5.30 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H).

Acetylation at room temperature overnight (Ac_2O–pyridine) gave the 3,18-diacetate (**39**) as a homogeneous (t.l.c.) but amorphous solid, $\nu_{max.}$ 1 730, 1 250, 1 205, 1 070, 1 040, and 870 cm^{-1}; δ 1.04 (s, 19-H_3), 1.34 and 1.46 (2 s, acetonide methyls), 2.05 (s, 3-OAc), 2.12 (s, 18-OAc), 4.12 (d, *J* 8 Hz, 17α-H), 4.17 and 4.27 (dd, *J* 12 Hz, 18-H_2), 4.66 (m, $w_{\frac{1}{2}}$ 16 Hz, 3α- and 16α-H), and 5.36 (m, $w_{\frac{1}{2}}$ 8 Hz, 6-H); mass spectrum, *m/z* (FAB) 447 $(M + H)^+$.

A by-product, homogeneous on h.p.l.c. obtained from the acetylation was identified as 16β,17β-isopropylidenedioxyandrost-5-ene-3β,18-diol 3-monoacetate (**40**), $\nu_{max.}$ 3 520, 1 730, 1 250, 1 200, 1 075, 1 060, 1 050, 1 030, and 870 cm^{-1}; δ 1.04 (s, 19-H_3), 1.34 and 1.56 (2 s, acetonide methyls), 2.05 (s, 3-OAc), 3.65 and 3.81 (dd, *J* 11 Hz, 18-H_2), 4.14 (d, *J* 8 Hz, 17α-H), 4.62 (m, $w_{\frac{1}{2}}$ 26 Hz, 3α-H and 16α-H), and 5.34 (m, $w_{\frac{1}{2}}$ 10 Hz, 6-H). Mass spectrum, *m/z* (FAB) 405 $(M + H)^+$.

* See footnote † p. 1827.

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