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16-SUBSTITUTED STEROIDS

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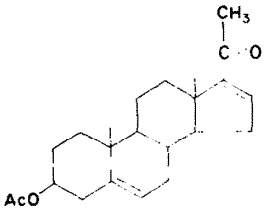
Abstract—The reactions of 3β-acetoxy-5,16-pregnadien-20-one with cyanide ion and with certain active methylene compounds are described. Subsequent transformations of the initial products to give various 16-substituted pregnenolones and progesterones are shown.

THE recent publication by Romo[1] of the addition of hydrocyanic acid to 16-pregnen-20-ones prompted us to record our own results with this and related reactions.[2] We have found that not only does 16-dehydropregnenolone acetate add hydrocyanic acid but that it also behaves as a typical α, β-unsaturated ketone in the Michael reaction. Thus, in the presence of strong alkali, we have prepared adducts with ethyl cyanoacetate, malononitrile, ethyl malonate,[3] ethyl acetoacetate and acetylacetone. The adducts were shown to be 16-substituted pregnenolones by infrared and ultraviolet spectra which demonstrated the presence of the 20-ketone and the absence of the conjugated Δ^{16}-20-ketone respectively.

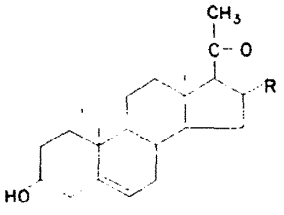
The addition of hydrocyanic acid to 16-dehydropregnenolone acetate (I) was carried out essentially as described by Romo[1] and 16α-cyano-3β-hydroxy-5-pregnen-20-one (II) was obtained. Michael reactions with I were run by two general methods. The first used ethanol as solvent and sodium ethoxide as the base. In this manner were obtained adducts III and IV with ethyl cyanoacetate and malononitrile respectively. Concomitant saponification of the 3-acetate took place. The second method used excess addend as solvent and preformed sodium enolate of the addend as base. The latter procedure yielded adducts V, VI and VII with ethyl malonate, ethyl acetoacetate and acetylacetone respectively. It should be noted that in the absence of solvent alcohol no saponification of the 3-acetate occurred. It was found possible to saponify III under mild conditions to the corresponding acid which was thermally decarboxylated yielding 16α-cyanomethyl-3β-hydroxy-5-pregnen-20-one (VIII), in effect the adduct of 16-dehydropregnenolone and acetonitrile. Oppenauer oxidation of adducts II, III, IV and VIII to the corresponding 16-substituted progesterones, IX, X, XI and XII, was straightforward and uniformly successful.

Some further transformations of 16α-cyanopregnenolone (II) were effected. Reduction of II with sodium borohydride gave 16α-cyano-5-pregnene-3β, 20β-diol (XIII) from which the diacetate XIV was prepared. The diol XIII was oxidized selectively to 16α-cyano-20β-hydroxy-4-pregnen-3-one (XV) by Oppenauer oxidation for a short time. Vigorous alkaline hydrolysis of diol XIII gave two products, a result of the expected epimerization at C-16 under the reaction conditions. The first was 16β-carboxy-5-pregnene-3β, 20β-diol 16, 20-lactone (XVII) characterized as the acetate

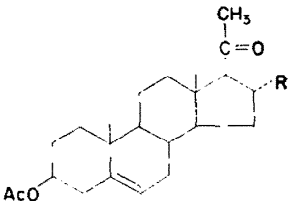
[1] J. Romo, *Tetrahedron* **3**, 37 (1958).
[2] R. H. Mazur, U.S. Pat. 2, 817, 671 (Dec. 24, 1957).
[3] P. Bladon, *J. Chem. Soc.* 3723 (1958).



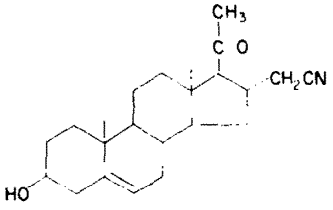
I



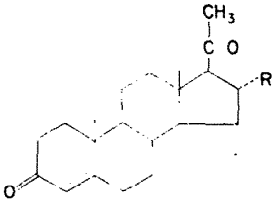
II R=CN
$CO_2C_2H_5$
III R=CHCN
IV R=$CH(CN)_2$



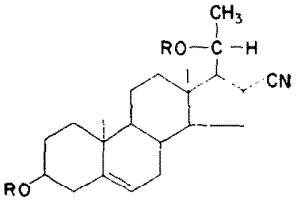
V R=$CH(CO_2C_2H_5)_2$
$CO_2C_2H_5$
VI R=CHCOCH$_3$
VII R=$CH(COCH_3)_2$



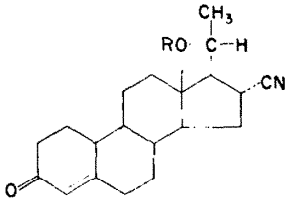
VIII



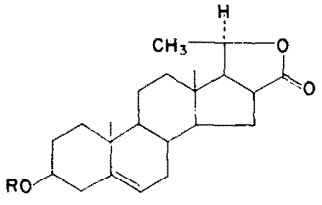
IX R=CN
$CO_2C_2H_5$
X R=CHCN
XI R=$CH(CN)_2$
XII R=CH_2CN



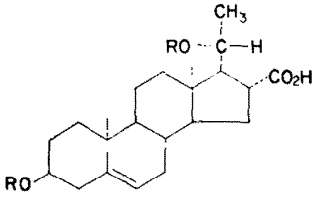
XIII R=H
XIV R=Ac



XV R=H
XVI R=Ac



XVII R=H
XVIII R=Ac



XIX R=H
XX R=Ac

XVIII and the second 16α-carboxy-5-pregnene-3β, 20β-diol (XIX) characterized as the diacetate XX. Compound XVII was never obtained as the hydroxyacid, lactonization being spontaneous. The configurations of XVII and XIX seem clear since such ready lactonization as XVII exhibits can only take place if the product has the two five-membered rings *cis*-fused. Oppenauer oxidation of XVII gave 16β-carboxy-20β-hydroxy-4-pregnen-3-one 16,20-lactone (XXI). The diacetate XX was saponified with two equivalents of sodium hydroxide, the resultant mixture (both XIX and the 20-monoacetate subsequently proved to be present) esterified with diazomethane and the crude esters subjected to Oppenauer oxidation. By a circuitous isolation procedure, very low yields of 20β-acetoxy-16α-carboxy-4-pregnen-3-one (XXII) and 16α-carbomethoxy-20β-hydroxy-4-pregnen-3-one (XXIII) were eventually obtained.

The assignment of the 20β-configuration to the diol XIII is based on molecular

rotation differences.[4,5] It may be seen from Table 1 that the molecular rotation changes upon acetylation of XIII to XIV and of XV to XVI are in agreement with the literature values for the acetylation of the corresponding 20β-hydroxy compounds without substituents at C-16.

A particularly interesting feature of the 1,4-addition reactions of 16-dehydropregnenolone acetate is that of stereochemistry. Previous chemists[6–11] have assumed the adducts to have the substituents in the 16α, 17β configuration (i.e. with a normal

TABLE 1

Compound	M_D	ΔM_D
5-Pregnene-3β,20α-diol[a]	−170	
5-Pregnene-3β,20α-diol diacetate	−216	−46
5-Pregnene-3β,20β-diol	−204	
5-Pregnene-3β,20β-diol diacetate	−145	+59
XIII[b]	−250	
XIV	−239	+11
20α-Hydroxy-4-pregnen-3-one	−312	
20α-Acetoxy-4-pregnen-3-one	−312	0
20β-Hydroxy-4-pregnen-3-one	+286	
20β-Acetoxy-4-pregnen-3-one	+462	+176
XV	+164	
XVI	+318	+154

[a] The reference compounds are described by R. B. Turner and D. M. Voitle, *J. Amer. Chem. Soc.* **73**, 2283 (1951). The rotations were measured in chloroform solution. [b] The rotations of XIII–XVI were measured in dioxane solution.

rather than an iso side chain at C-17), an assignment which follows from the approach of the anion on the least hindered side of the molecule. We feel there is little reason to doubt this interpretation of the reaction. In the present work, also, the substituents at C-16 very probably have the 16α configuration although the molecular rotation changes are erratic and permit no definite conclusions. A further complicating factor in compounds III and VI is the introduction of a new asymmetric centre in the side chain. That none of the adducts has a 16α,17α or 16β,17α configuration is shown, we feel, by the fact that not one product has a negative rotation. Additionally, oxidation of our 16-substituted pregnenolones yielded 16-substituted progesterones showing large positive specific rotations but not substances having low specific rotations. As it is known that 3β-hydroxy-5α,17α-pregnan-20-one[12] and 3β-hydroxy-17α-pregn-5-en-20-one[13] have large negative specific rotations while 17α-pregn-4-ene-3,20-dione (17-isoprogesterone)[14] has a zero rotation, it seems quite certain that

[4] L. F. Fieser and M. Fieser, *Experientia* **4**, 285 (1948).
[5] L. H. Sarett, *J. Amer. Chem. Soc.* **71**, 1175 (1949).
[6] D. K. Fukushima and T. F. Gallagher, *J. Amer. Chem. Soc.* **73**, 196 (1951).
[7] J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *J. Amer. Chem. Soc.* **73**, 1528 (1951).
[8] H. Hirschmann, E. B. Hirschmann and M. A. Daus, *J. Amer. Chem. Soc.* **74**, 539 (1952).
[9] D. Gould, F. Gruen and E. B. Hershberg, *J. Amer. Chem. Soc.* **75**, 2510 (1953).
[10] G. P. Mueller and B. Riegel, *J. Amer. Chem. Soc.* **76**, 3686 (1954).
[11] D. Gould, E. L. Shapiro, L. E. Finckenor, F. Gruen and E. B. Hershberg, *J. Amer. Chem. Soc.* **78**, 3158 (1956).
[12] C. W. Shoppee, *J. Chem. Soc.* 1671 (1949).
[13] A. Butenandt and G. Fleischer, *Ber.* **70**, 96 (1937).
[14] A. Butenandt, J. Schmidt-Thomé and H. Paul, *Ber.* **72**, 1112 (1939).

the substances prepared by anionic addition to 16-dehydropregnenolone acetate have 17β side chains.[15] It can of course be argued that introduction of a new asymmetric centre at C-16 vitiates any conclusions which may be drawn from unsubstituted steroids. However, it would be most amazing if a vicinal effect could completely nullify the very large 17-iso shift. For example, were 16-cyanopregnenolone to be, in fact, 16-cyano-17-isopregnenolone, introduction of the cyano group (whether α or β) would require a molecular rotary shift of around $+500°$.

An additional interesting aspect is that a 16-substituted pregnenolone *was* obtained[1,16] having a large negative specific rotation and yielding a 16-substituted progesterone having a low positive specific rotation. Further, these compounds were prepared[1] under conditions known[12,13] to isomerize the 17β-to the 17α-acetyl side chain. Specifically, strong alkaline hydrolysis[1] of 16α-cyanopregnenolone gave a 16-carboxypregnenolone with an attendant molecular rotatory shift of $-463°$ (compare the change XIV to XX with $\Delta M_D + 3°$). Similar hydrolysis[1] of 16α-cyanoprogesterone produced a 16-carboxyprogesterone and a molecular rotatory shift of $-479°$.[17] We feel this typical rotatory shift indicates that isomerization at C-17 has occurred and we believe that inversion at C-16 has happened also. Reduction[1] of 3β-acetoxy-16β-carbomethoxy-17α-pregn-5-en-20-one (XXV) with sodium borohydride and subsequent alkaline saponification yielded 16β-carboxy-17α-pregn-5-ene-3β,20-diol (XXVI). The latter could not be induced to cyclize even under forcing conditions. Romo[1] interpreted this result as proving that the 16-carboxyl and *20-hydroxyl* were *trans*. Rather, failure to cyclize means that the 16-carboxyl and 17-hydroxy-ethyl *side-chain* are *trans*. If we assume the side chain to have the 17α configuration, then the carboxyl group must be 16β. The most likely reaction during which the group at C-16 could have isomerized is saponification of the nitrile. Thus, the alkaline saponification[1] of 16α-cyanopregnenolone and 16α-cyanoprogesterone very likely gave 16β-carboxy-3β-hydroxy-17α-pregn-5-en-20-one (XXIV) and 16β-carboxy-17α-pregn-4-ene-3,20-dione (XXVIII) respectively.

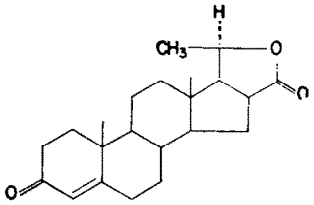
We may say in general that at equilibrium the configuration of a 16-substituted 20-ketopregnane is determined by the steric requirements of the group at C-16. Under alkaline conditions, one obtains 16α-cyanopregnenolone but 16β-carboxy-17-isopregnenolone. Thus, the linear cyano group can be accommodated in the 16α position giving the product expected from backside attack on ring D. However, the preferred orientation of the bulkier carboxyl group is apparently 16β leading (in the presence of an easily epimerizable acetyl group) to the 17α-acetyl side chain which latter arrangement is associated with less steric hindrance among the groups at C-13, 16 and 17.

A coincidence is exhibited by XX and the substance XXVII to which Romo[1] assigned the same structure but which most likely has the opposite configuration at both C-16 and C-17. The two compounds differ greatly in melting point but have

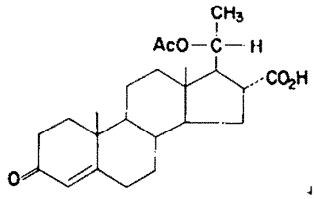
[15] As a point of internal consistency, 3β-hydroxy-5α,17α-pregnan-20-one, 3β-hydroxy-17α-pregn-5-en-20-one and 17-isoprogesterone show large negative and approximately equal molecular rotatory shifts relative to the appropriate 17β (normal) isomers. The values of ΔM_D(17α-17β) are $-532°$, $-533°$ and $-605°$ respectively.

[16] B. Ellis, V. Petrow and D. Wedlake, *J. Chem. Soc.* 3748 (1958). These authors also studied the reaction of potassium cyanide with 16-dehydropregnenolone and hydrolysis of the resulting 16-cyanopregnenolone. They obtained, among others, compounds II, XXIV and XXVIII and assigned the same structures as did Romo.[1] The arguments in the present paper, therefore, apply equally to the results of Ellis *et al.*

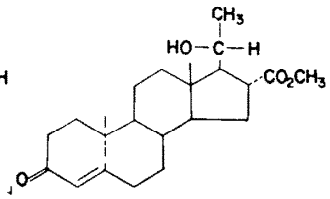
[17] The same substance was obtained by Oppenauer oxidation of the above 16-carboxypregnenolone.[1]



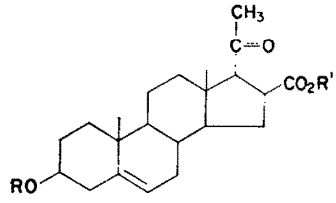
XXI



XXII

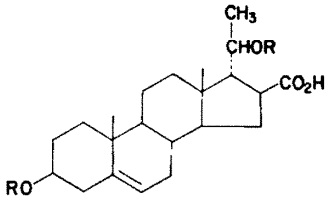


XXIII



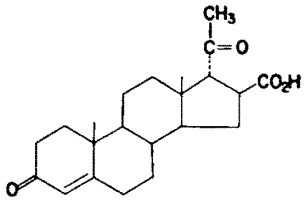
XXIV R = R' = H

XXV R = Ac, R' = CH_3



XXVI R = H

XXVII R = Ac



XXVIII

nearly identical rotations. The changes in molecular rotation for the steps II → XIII → XX and II → XXIV → XXVI → XXVII are shown in Table 2. The large negative shift in the reduction of II to XIII is in accord with other reductions of 20-keto-17β-pregnanes.[4,5] The large negative molecular rotatory shift caused by the hydrolysis of II to XXIV already has been shown to be due principally to inversion at C-17. The moderately large positive shift associated with the conversion of XXIV to XXVI may prove to be generally typical of the reduction of a 20-keto-17α-pregnane. At present, there are no other examples in the literature of 20-hydroxy compounds in the 17α series (without an oxygen function at C-17 also). The positive change in molecular rotation upon acetylation of XXVI to XXVII may indicate that XXVI has a 20β-hydroxyl group, but the lack of suitable models prevents a definite assignment of configuration.

TABLE 2

Compound	M_D[a]	ΔM_D[d]
II	+78 (M),[b] +48 (C)[c]	
IX	+540 (C)[c]	
XIII	−250 (D)[b]	−328 (II M)
XX	−236 (D)[b]	+14 (XIII)
XXIV	−415 (C)[c]	−463 (II C)
XXVI	−306 (D)[c]	+109 (XXIV)
XXVII	−239 (C)[c]	+67 (XXVI)
XXVIII	+61 (C)[c]	−479 (IX)

[a] The solvent for rotation measurement is indicated by C = chloroform, D = dioxane and M = methanol. [b] Present work. [c] Reference 1. [d] The numeral in parentheses shows the reference compound.

EXPERIMENTAL[18]

16α-Cyano-3β-hydroxy-5-pregnen-20-one (II). 16-Dehydropregnenolone acetate (20·2 g, 0·057 mole) and 20 g (0·31 mole) potassium cyanide were suspended in 400 ml methanol, 40 ml ethyl acetate and 40 ml water. The mixture was heated at the reflux temp for 2 hr during which time a clear solution resulted. Dilution with 500 ml water and cooling yield 17·1 g crude product. Crystallization from benzene–ethanol by concentrating to remove ethanol gave 13·2 g (68%) needles, m.p. 225–228°. Recrystallization from butyl acetate raised the m.p. to 231–234°; $[\alpha]_D$ +23° (*c* 0·5, methanol) (Found: C, 77·49; H, 8·97; N, 4·04. Calc. for $C_{22}H_{31}NO_2$: C, 77·38; H, 9·15; N, 4·10%).

16α-(α-Carbethoxycyanomethyl)-3β-hydroxy-5-pregnen-20-one (III). To a solution of 14 g (0·6 mole) sodium in 1 l. dry ethanol was added 68 g (0·6 mole) ethyl cyanoacetate and 107 g (0·3 mole) 16-dehydropregnenolone acetate. The mixture was stirred until solution occurred (ca. 1½ hr) and allowed to stand overnight at room temp. The solution was neutralized with acetic acid, diluted with chloroform and washed with dil HCl and water until no further color was extracted. The residue from distillation of the chloroform yielded 34 g crude III on trituration with ethyl acetate. Crystallization of this crude product from ethyl acetate gave 27·0 g tiny needles, m.p. 198–200°. The combined mother liquors were chromatographed on silica gel. Elution with 20% ethyl acetate–benzene and subsequent crystallization from ethyl acetate gave an additional 31·0 g of the desired product, m.p. 196–198°; yield, 58·0 g (45%); $[\alpha]_D$ +18° (*c* 1·0, methanol) (Found: C, 72·93; H, 8·51; N, 3·23. $C_{26}H_{37}NO_4$ requires: C, 73·03; H, 8·72; N, 3·28%).

16α-Dicyanomethyl-3β-hydroxy-5-pregnen-20-one (IV). The above procedure was followed using 1·4 g (0·06 mole) of sodium, 4·0 g (0·06 mole) malononitrile and 10·7 g (0·03 mole) 16-dehydropregnenolone acetate. The crude product was chromatographed on silica gel. Elution with 20% ethyl acetate–benzene and crystallization of the material thus obtained from benzene gave 3·8 g (33%), m.p. 203–207°. Recrystallization from 95% ethanol raised the m.p. to 206–209°; $[\alpha]_D$ 0° (*c* 1·0, chloroform) (Found: C, 75·78; H, 8·63; N, 7·10. $C_{24}H_{32}N_2O_2$ requires: C, 75·75; H, 8·48; N, 7·36%).

3β-Acetoxy-16α-dicarbethoxymethyl-5-pregnen-20-one (V). To 1·5 g (0·065 mole) sodium in 100 ml diethyl malonate was added a suspension of 17·8 g (0·05 mole) 16-dehydropregnenolone acetate in 100 ml diethyl malonate. The steroid was dissolved by brief warming and the solution allowed to stand overnight at room temp. The solution was acidified with acetic acid, diluted with ether and washed well with water. Distillation of the organic solvents left a solid residue which was chromatographed on silica gel. Elution with 10% ethyl acetate-benzene yielded 14·1 g crude material from which was obtained by two crystallizations from 70% ethanol 9·0 g (35%) of the desired product, m.p. 122–124°; $[\alpha]_D$ +43° (*c* 0·9, dioxane) (Found: C, 69·86; H, 8·54. $C_{30}H_{44}O_7$ requires: C, 69·74; H, 8·58%).

3β-Acetoxy-16α-(α-carbethoxyacetylmethyl)-5-pregnen-20-one (VI). The above procedure with identical quantities was followed except that ethyl acetoacetate was substituted for diethyl malonate. The desired product was eluted from silica gel with 10% ethyl acetate–benzene. Crystallization from methanol gave VI, 12·7 g (52%), m.p. 163–167°. Recrystallization from isopropyl ether raised the m.p. to 165–167°; $[\alpha]_D$ +28° (*c* 1·1, dioxane) (Found: C, 71·50; H, 8·59. $C_{29}H_{42}O_6$ requires: C, 71·57; H, 8·70%).

3β-Acetoxy-16α-diacetylmethyl-5-pregnen-20-one (VII). The above procedure was repeated using acetylacetone in place of ethyl acetoacetate. Trituration of the crude product with methanol resulted in the recovery of 11·0 g of starting material. Chromatography of the remainder and elution with 10% ethyl acetate–benzene yielded after crystallization from ethanol, 0·80 g (9%) of VII, m.p. 182–186°; $[\alpha]_D$ −86° (*c* 1·0, dioxane) (Found: C, 73·71; H, 8·72. $C_{28}H_{40}O_5$ requires: C, 73·68; H, 8·77%).

16α-Cyanomethyl-3β-hydroxy-5-pregnen-20-one (VIII). Adduct III (10·6 g, 0·025 mole) and 1·7 g (0·03 mole) potassium hydroxide in 106 ml dioxane and 30 ml water were heated on the steam bath for 45 min. The solution was acidified with acetic acid, diluted with water and concentrated to remove most of the dioxane. The product was extracted with chloroform. Distillation of the chloroform gave the cyanoacetic acid derivative, m.p. ca. 240° with gas evolution. The crude acid was decarboxylated by heating at 275–300° until carbon dioxide evolution ceased (ca. ½ hr). Crystallization of the residue from ethyl acetate yielded small needles, 8·3 g (94%), m.p. 202–204°. Recrystallization from ethyl

[18] We would like to thank Robert T. Dillon and associates for analyses. Melting points are uncorrected. Rotations were determined at 25 ± 3°. Ultraviolet spectra were carried out in methanol.

acetate raised the m.p. to 206–207°; $[\alpha]_D$ +14° (*c* 1·1, chloroform) (Found: C, 77·64; H, 9·03; N, 3·88. $C_{23}H_{33}NO_2$ requires: C, 77·70, H, 9·36; N, 3·94%).

Oppenauer oxidations. The general procedure followed was to dissolve the compound in 40–50 vol toluene and 5 vol freshly redistilled cyclohexanone. Five vol 20% aluminum isopropoxide in toluene were added and the solution heated under reflux for 2 hr. To the cooled solution was added an equal vol of 50% aqueous potassium sodium tartrate and the organic solvents removed by extensive steam distillation. The product was extracted with benzene, chromatographed on silica gel and eluted with 10% ethyl acetate–benzene.

Adduct II (2·98 g) gave 16α-*cyanoprogesterone* (IX), 1·72 g (57%), prisms from aqueous methanol, m.p. 230–233°; $[\alpha]_D$ +151° (*c* 0·5, methanol); λ_{max} 240 mμ, ε = 17,300 (Found: C, 77·89; H, 8·54; N, 4·07. Calc. for $C_{22}H_{29}NO_2$: C, 77·83; H, 8·61; N, 4·13%).

Adduct III (3·24 g) gave 16α-(α-*carbethoxycyanomethyl*)-*progesterone* (X), 1·22 g (37%), needles from ethyl acetate–cyclohexane, m.p. 213–216°; $[\alpha]_D$ +103° (*c* 0·5, methanol); λ_{max} 240 mμ, ε = 16,500 (Found: C, 73·41; H, 8·22; N, 3·29. $C_{26}H_{35}NO_4$ requires: C, 73·38; H, 8·29; N, 3·29%).

Adduct IV (1·75 g) gave 16α-*dicyanomethylprogesterone* (XI), 1·11 g (64%), prisms from benzene–cyclohexane, m.p. 206–210°; $[\alpha]_D$ −112° (*c* 1·0, chloroform); λ_{max} 240 mμ, ε = 16,300 (Found: C, 76·40; H, 7·99; N, 6·93. $C_{21}H_{30}N_2O_2$ requires: C, 76·15; H, 7·99; N, 7·40%).

Adduct VIII (52·0 g) gave 16α-cyanomethylprogesterone (XII), 31·3 g (60%), prisms from benzene–cyclohexane, m.p. 185–186°; $[\alpha]_D$ +142° (*c* 0·5, methanol); λ_{max} 240 mμ, ε = 16,300 (Found: C, 78·35; H, 8·95; N, 4·02. $C_{23}H_{31}NO_2$ requires: C, 78·13; H, 8·84; N, 3·96%).

16α-*Cyano*-5-*pregnene*-3β,20β-*diol* (XIII). Cyanopregnenolone II (6·0 g 0·0175 mole) was dissolved in 100 ml hot ethanol. Sodium borohydride (1·5 g) in 15 ml water was added and the solution warmed on the steam bath for ½ hr. The solution was acidified with acetic acid and concentrated to a small vol yielding 5·0 g (83%) XIII, m.p. 224–228°. Crystallization from methanol raised the m.p. to 231–233°; $[\alpha]_D$ −73° (*c* 0·5, dioxane) (Found: C, 76·78; H, 9·54. $C_{22}H_{33}NO_2$ requires: C, 76·92; H, 9·68%).

The *diacetate* XIV, m.p. 212–213° (methanol), was prepared by brief refluxing of XIII with pyridine and acetic anhydride; $[\alpha]_D$ −56° (*c* 0·5, dioxane) (Found: C, 72·99; H, 8·52. $C_{26}H_{37}NO_4$ requires: C, 73·03; H, 8·72%).

16α-*Cyano*-20β-*hydroxy*-4-*pregnen*-3-*one* (XV). The general procedure for Oppenauer oxidations was used except that the solution was heated under reflux for only 10 min. The crude material from 2·0 g XIII was chromatographed on silica gel. Elution with 25% ethyl acetate–benzene and subsequent crystallization from benzene gave the desired product, 0·41 g (20%), m.p. 215–219°; $[\alpha]_D$ +48° (*c* 1·0 dioxane); λ_{max} 240·5 mμ, ε = 16,500 (Found: C, 77·33; H, 8·83. $C_{22}H_{31}NO_2$ requires: C, 77·37; H, 9·15%).

The *acetate* XVI, m.p. 217–218° (methanol), was prepared by treatment with acetic anhydride in pyridine overnight at room temp; $[\alpha]_D$ +83° (*c* 1·0 dioxane); λ_{max} 240 mμ, ε = 17,200 (Found: C, 75·17; H, 8·69; N, 3·51. $C_{24}H_{33}NO_3$ requires: C, 75·16; H, 8·67; N, 3·65%).

Saponification of XIII. 16α-Cyano-5-pregnene-3β,20β-diol (38·0 g, 0·11 mole) in 1 l. of 95% ethanol was treated with 200 g potassium hydroxide in 500 ml water and the solution heated under reflux for 3 days. Acidification of the cooled solution with HCl gave a gelatinous product. The crude material was acetylated by heating with pyridine and acetic anhydride and the mixture of acetates chromatographed on silica gel. Elution with 5% ethyl acetate–benzene yielded, after crystallization from ethanol, 8·5 g (20%) of 3β-*acetoxy*-20β-*hydroxy*-5-*pregnen*-16β-*carboxylic acid* 16,20-*lactone* (XVIII), m.p. 238–240°; $[\alpha]_D$ −35° (*c* 1·0, dioxane); λ_{max}^{KBr} 5·67, 5·81 μ (Found: C, 74·52; H, 8·88. $C_{24}H_{34}O_4$ requires: C, 74·58; H, 8·87%).

Alkaline hydrolysis of XVIII yielded, after acidification to reclose the lactone, 16β-*carboxy*-5-*pregnene*-3β,20β-*diol* 16,20-*lactone* (XVII), m.p. 241–243° from ethyl acetate; $[\alpha]_D$ −31° (*c* 0·6, dioxane) (Found: C, 76·47; H, 9·76. $C_{22}H_{32}O_3$ requires: C, 76·70; H, 9·34%).

Elution of the column with 10% ethyl acetate–benzene and subsequent crystallization from ethyl acetate–cyclohexane gave 14·0 g (29%) of 3β,20β-*diacetoxy*-5-*pregnene*-16α-*carboxylic acid* (XX), m.p. 184–185°; $[\alpha]_D$ −53° (*c* 0·8, dioxane) (Found: C, 69·92; H, 8·53. $C_{26}H_{38}O_6$ requires: C, 69·92; H, 8·85%).

Saponification of XX resulted in 16α-*carboxy*-5-*pregnene*-3β,20β-*diol* (XIX), m.p. 289–293° from ethanol (Found: C, 71·35; H, 9·40. $C_{22}H_{34}O_4 \cdot \frac{1}{2}C_2H_5OH$ requires: C, 71·65; H, 9·67%).

16β-*Carboxy*-20β-*hydroxy*-4-*pregnen*-3-*one* 16,20-*lactone* (XXI). Oppenauer oxidation of XVII

by the general procedure described above—except that the solution was heated under reflux for 15 min—yielded the desired product after chromatography on silica gel and elution with 20% ethyl acetate–benzene. From 0·90 g XVII was obtained 0·55 g (60%) XXI, m.p. 188–190° from ethyl acetate–petroleum ether (b.p. 60–80°); $[\alpha]_D$ +96° (*c* 1·0, dioxane); λ_{max} 241 mμ, ε − 17,000 (Found: C, 77·49; H, 8·97. $C_{22}H_{30}O_3$ requires: C, 77·15; H, 8·83%).

20β-Acetoxy-16α-carboxy-4-pregnen-3-one (XXII) *and 16α-carbomethoxy-20β-hydroxy-4-pregnen-3-one* (XXIII). Compound XX (4·46 g, 0·01 mole) was allowed to stand overnight at room temp with a solution of 0·46 g (0·02 mole) sodium in 200 ml 90% ethanol. The solution was concentrated to a small vol, diluted with water and acidified with HCl, The voluminous precipitate was collected by filtration, washed with water and dried. The crude product was suspended in 30 ml dioxane and esterified with ethereal diazomethane. The resultant solution was concentrated to dryness and oxidized directly by the usual Oppenauer procedure with the reaction time limited to 15 min. The crude product was chromatographed on silica gel. The material eluted with 10% ethyl acetate–benzene was saponified with aqueous methanolic sodium hydroxide, acetylated with acetic anhydride in pyridine and rechromatographed on silica gel to yield 20β-acetoxy-16α-carboxy-4-pregnen-3-one (XXII), 0·25 g, m.p. 239–243°, from ethyl acetate–isopropyl ether; $[\alpha]_D$ +66° (*c* 0·5, dioxane); λ_{max} 241, ε − 16,400 (Found: C, 71·75; H, 8·66. $C_{24}H_{34}O_5$ requires: C, 71·61; H, 8·51%).

The fraction eluted from the original column with 30% ethyl acetate–benzene gave 16α-carbomethoxy-20β-hydroxy-4-pregnen-3-one (XXIII), 0·05 g, m.p. 144–146° from petroleum ether (b.p. 60–80°); $[\alpha]_D$ +31° (*c* 1·0, dioxane); λ_{max} 241, ε − 15,700 (Found: C, 73·81; H, 9·26. $C_{23}H_{34}O_4$ requires: C, 73·75; H, 9·10%).