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18-SUBSTITUTED STEROIDS: SYNTHESIS OF 18-HYDROXYCORTISOL (11β,17α,18,21-TETRAHYDROXY-4-PREGNENE-3,20-DIONE) AND 18-HYDROXYCORTISONE (17α,18,21-TRIHYDROXY-4-PREGNENE-3,11,20-TRIONE)

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Summary—The isolation of 18-hydroxycortisol from the urine of patients with primary aldosteronism was recently described and no synthetic procedure was available for its preparation. The C-13 angular methyl group of prednisolone-17α,21-acetonide-11β-nitrite was functionalized by photolysis in the presence of oxygen to give the product 18-hydroxy-prednisolone-17α,21-acetonide-18-nitrate. The 18-nitrate was reduced with zinc and ammonium acetate to the corresponding 18-hydroxy compound, 18-hydroxy-prednisolone-17α,21-acetonide. Homogeneous hydrogenation with Tris(triphenyl-phosphine)rhodium (I) chloride as catalyst resulted in the formation of 18-hydroxy-cortisol-17α,21-acetonide. Acid hydrolysis of the latter compound gave 18-hydroxycortisol. Oxidation of 18-hydroxycortisol-17α,21-acetonide with pyridinium dichromate followed by acid hydrolysis gave 18-hydroxycortisone. The 18-hydroxylated steroids exist as the 18,21-hemiacetals. Catalytic reduction with tritium gas with Tris(triphenyl-phosphine)rhodium (I) chloride of 18-hydroxyprednisolone-17α,21-acetonide and acid hydrolysis gave [1,2^{3}H]18-hydroxycortisol.

INTRODUCTION

The isolation and identification of 18-hydroxycortisol from the urine of patients with adrenal adenomas producing primary aldosteronism was recently described [1]. Recent studies suggest that cortisol can be metabolized through the same metabolic pathway as that of corticosterone to aldosterone, with the formation of 18-hydroxycortisol and 18-oxocortisol [1, 2]. The study of the biosynthesis, function and role in disease processes of 18-hydroxycortisol has been hindered by the unavailability of this compound. There are several procedures described for the synthesis of 18-hydroxylated pregnanes most of which are based on the photochemical activation of the angular methyl group either by the hypoiodite reaction [3, 4] or photolysis of nitrites under oxygenating conditions [5]. The synthesis of 18-hydroxycortisol presented special problems because of the presence of the 17α-hydroxy group. The side chain was not expected to survive the hypoiodite reaction since lead tetraacetate oxidizes and cleaves the side chain of the 17α,20β-dihydroxy derivative that would have to be used as a precursor [6]. We describe a method for the synthesis of 18-hydroxycortisol using principles similar to the one described by Barton *et al.*[5] for the synthesis of 18-hydroxycorticosterone. The starting material was the 1,4-dien-3-one steroid, prednisolone, since the distance between the 11β-hydroxy and the C-13 angular methyl groups would favor the almost exclusive functionalization of the latter group [7] rather than that of the C-19 methyl group. Protection of the dihydroxyacetone side chain was achieved by preparing the 17α,21-acetonide, which can be easily hydrolyzed thereafter with dilute mineral acid [8]. Treatment of the acetonide with nitrosyl chloride in pyridine afforded the corresponding 11β-nitrite (**II**). Irradiation of the 11β-nitrite under oxygen produced the 18-nitrate (**III**). Reduction of the C-18 nitrate with zinc and ammonium acetate in methanol gave the C-18 alcohol (**IV**). Hydrogenation of the 18-hydroxy-prednisolone acetonide (**IV**) with hydrogen gas in the presence of the catalyst Tris(triphenylphosphine)rhodium (**I**) chloride [9] produced 18-hydroxycortisol-17α,21-acetonide (**V**). A similar reaction done with carrier-free tritium gas provided the [1,2^{3}H]18-hydroxycortisol-17α,21-acetonide of high specific activity. Acid hydrolysis of the acetonides resulted in the formation

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of 18-hydroxycortisol (**VI**) and [1,2^{3}H]18-hydroxycortisol respectively. Oxidation of 18-hydroxycortisol-17α,21-acetonide with pyridinium dichromate in dichloromethane produced 18-hydroxycortisone-17α,21-acetonide (**VII**). Acid hydrolysis of the acetonide yielded 18-hydroxycortisone (**VIII**).

MATERIALS AND METHODS

Prednisolone was purchased from Sigma Chemical Co. (St Louis, MO). Nitrosyl chloride was purchased from Matheson Co. (East Rutherford, NJ). Other reagent grade chemicals and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). The photochemical reactor with a 400 watt Hanovia lamp was manufactured by Ace Glass (Vineland, NJ). Melting points were determined on a Koeffler hot stage melting point apparatus. I.r. spectra were determined for KBr discs on a Pye-Unicam SP3-200 spectrophotometer. Mass spectra were recorded on a Finnigan Model 4021 automated Electron Impact/Chemical Ionization Gas Chromatograph, Mass Spectrometer (Finnigan Instruments, Palo Alto, CA) coupled to an INCOS 2000 data processing system. Mass spectra were obtained at temperatures ranging between 100–150°C. Electron impact ionization (30 electron volts energy) was employed and spectra were recorded by automatic repetitive scanning over the mass range 0–500 a.m.u. The samples were introduced using the direct inlet probe. Nuclear magnetic resonance (NMR) spectra, with tetramethylsilane as internal standard, were determined either at 100 MHz on a Jeol JNM-FX100 spectrometer or at 400 MHz on the University of London Intercollegiate Research Service Bruker WH-400 spectrometer at Queen Mary College.

Synthesis of prednisolone-17α,21-*acetonide* (11β,17α,21-*trihydroxy*-1,4-*pregnadiene*-3,20-*dione* 17α,21-*acetonide*) (**I**)

This compound was prepared as reported (8); it had m.p. 240–242°C (lit, (8) 243–247°C); i.r.: ν_{max} 3485 (O-H), 1711 (20-CO), 1654, 1610, 1600 sh and 890 cm^{-1} (1,4-dien-3-one); NMR: $\delta(CDCl_3)$ 0.95 (s, 18-H_3), 1.40, 1.44 and 1.48 (s,s,s, 19-H_3 and acetonide-H_6), 4.28 and 4.35 (dd, $J = 18$ Hz, 21-H_2), 4.51 (m, $W = 8$ Hz, 11α-H), 6.03 (br s, $W \simeq 4$ Hz, 4-H), 6.29 (dd, $J_{1,2} = 19$ Hz, $J_{2,4} \simeq 1.5$ Hz, 2-H), and 7.31 (d, $J_{1,2} = 10$ Hz, 1-H); m.s.: the molecular ion (M^+) is at mass per unit charge (m/z) 400, prominent ions are seen at m/z 342 (M^+-58, loss of acetone) m/z 324, 300, 249, 223, 207, 161, 147, 135, 122, 121, 72, 58. the base peak is at m/z 121.

Synthesis of prednisolone-17α,21-*acetonide*-11β-*nitrite* (11β,17α,21-*trihydroxy*-1,4-*pregnadiene*-3,20-*dione*-17α,21-*acetonide*-11β-*nitrite*) (**II**)

Prednisolone-17α,21-acetonide (**I**) [10.0 g] was dissolved in pyridine (200 ml) and the solution was chilled in an ice-water slurry. Nitrosyl chloride was bubbled through the solution until it underwent a color change to dark brown. The solution was poured on an ice-water slurry to give a precipitate that was collected by filtration. After thorough washing with water, product was dried at room temperature in the dark. The product (10.1 g), prednisolone-17α,21-acetonide 11β-nitrite-(**II**) was better than 95% pure, as demonstrated by thin layer chromatography (TLC) on silica gel GF_{254} plates developed with a solvent system made of chloroform–isopropanol (97:3, v/v). this material decomposes spontaneously on storage within a few days even at 0°C. Therefore, compound **II** was used immediately after preparation without further purification. The 11β-nitrite (**II**), had m.p. 134–136°C, m.s.: the M^+ is at m/z 429; prominent ions are at m/z 371 (M^+-58, loss of acetone), m/z 342, 324, 302, 281, 255, 238, 223, 211, 185, 173, 161, 147, 135, 122, 121, 72, 58. The base peak is at m/z 121; NMR: the material decomposed rapidly in $CDCl_3$ but more slowly in C_5D_5N, where it was possible to observe the following signals along with those of decomposition products: δ, 1.08 (s, 18-H_3), 1.32, 1.38, 1.42 (s,s,s, 19-H_3 and acetonide-H_6), 6.22 (br.s, w ≃ 6 Hz, 4-H), *ca* 4.66 (m, 11α-H) overlapping 6.45 (dd, $J_{1,2} = 10$ Hz, $J_{2,4} \simeq 1$ Hz, 2-H) and 7.46 (d, $J_{1,2} = 10$ Hz, 1-H).

Synthesis of 18-*hydroxy*-*prednisolone*-17α,21-*acetonide*-18-*nitrate* (11β,17α,18,21-*tetrahydroxy*-1,4-*pregnadiene*-3,20-*dione*-17α,21-*acetonide*-18-*nitrate*) (**III**)

Freshly prepared 11β-nitrite (**II**) [9.0 g] was dissolved in a mixture of acetonitrile (800 ml) and triethylamine (3 ml). The solution was cooled at −10°C, oxygen was bubbled through it and the mixture was irradiated (Pyrex filter) with a 400 W Hanovia lamp for 90 min. The solvent was evaporated under vacuum at 45°C. An aliquot (2.0 g) of the crude product was purified by use of preparative high-performance liquid chromatography (HPLC) on a 4 × 40 cm column filled with silica gel H (10 ± 4 μm particles). A linear gradient of dichloromethane (100%) and dichloromethane–isopropanol (up to 10%) at a flow rate of 10 ml/min, over a period of 4 h, was used for elution. Fractions containing the 18-nitrate (**III**) were pooled, evaporated to dryness and crystallized (from acetone), m.p. 180–182°C; i.r.: ν_{max} 3320 (O-H), 1712 (2O-CO), 1654, 1610, 1600 and 890 (1,4-dien-3-one), 1620 and 1280 cm^{-1} (ONO_2): NMR: $\delta(CDCl_3)$ 1.40, 1.44, 1.46, (s,s,s, 19-H_3 and acetonide-H_6), 4.34 (s, 21-H_2), 4.29 and 4.93 (dd, $J = 10$ Hz, 18-H_2), 4.56 (m, $W = 8$ Hz, 11α-H), 6.04 (br s, $W \simeq 4$ Hz, 4-H), 6.30 (dd, $J_{1,2} = 10$ Hz, $J_{2,4} \simeq 1.5$ Hz, 2-H), and 7.27 (d, $J_{1,2} = 10$ Hz, 1-H); m.s.: the M^+ is at 461: prominent ions are at m/z 479, 415, 403, 398, 380, 356, 340, 311, 297, 267, 237, 207, 171, 160, 147, 135, 122, 121, 72. The base peak is at m/z 58 (acetone).

Synthesis of 18-*hydroxyprednisolone* 17α,21-*acetonide* (11β,17α,18,21-*tetrahydroxy*-1,4-*pregnadiene*-3,20-*dione*-17α,21-*acetonide*) (**IV**)

The remainder of the crude nitrate **III** was dissolved in methanol (100 ml), ammonium acetate (2.0 g) and zinc dust (10.0 g) were added and the mixture was stirred at 24°C for 30 min. The suspension was filtered and the cake was repeatedly washed with methanol. The pooled methanol fractions were evaporated, in part, at 45°C *in vacuum* and then taken to 1.5 l with dichloromethane. The dichloromethane solution was washed sequentially with water, 5% sodium bicarbonate solution and water, and then evaporated to dryness *in vacuum*. The 18-hydroxy product (**IV**) was purified by chromatography on Florisil (200 g), eluting with a gradient made of increasing concentrations of isopropanol in dichloromethane. The product was crystallized from acetone–hexane and the final yield was 1.5 g, m.p. 236–240°C; i.r.: 3420 (broad, O-H), 1655, 1612, 1600 sh and 892 cm^{-1} (1,4-dien-3-one); NMR: $\delta(CDCl_3)$ 1.24, and 1.40 (s,s, acetonide-H_6), 1.44 (s, 19-H_3), 3.64 (s, 21-H_2), 3.65 and 4.75 (dd, $J = 9$ Hz, 18-h_2), 4.52 (m, $W \simeq 8$ Hz, 11α-H), 6.02 (br, s, $W \simeq 4$ Hz, 4-H), 6.29 (dd, $J_{1,2} = 10$ Hz, $J_{2,4} \simeq 1.5$ Hz, 2-H), and 7.30 (d, $J_{1,2} = 10$ Hz, 1-H); m.s.: the M^+ was not detected; prominent ions are at *m/z* 398 (M^+-18, loss of H_2O, *m/z* 385 ($M^+ - 31$, loss of CH_2-OH), *m/z* 340 ($M^+ - 76$, loss of H_2 plus acetone); prominent ions are at *m/z* 311, 297, 265, 251, 249, 237, 223, 205, 171, 169, 147, 134, 122, 121, 72. The base peak is at *m/z* 58 (acetone).

Synthesis of 18-*hydroxycortisol* 17α,21-*acetonide* (11β,17α,18,21-*tetrahydroxy*-4-*pregn*-3,20-*dione*-17α,21-*acetonide*) (**V**)

18-Hydroxy-prednisolone 17α,21-acetonide (**IVd**) (750 mg) was dissolved in acetone (100 ml), Tris(triphenylphosphine)rhodium (**I**) chloride (500 mg) was added, and the solution was exposed to hydrogen at 1 atom at 24°C for 3 h. Complete hydrogenation of the 1,2-double bond was demonstrated by use of TLC on silica gel GF_{254} with the developing system made of isopropyl ether–diozane (4:1, v/v). The solution was filtered and partially evaporated *in vacuum*. Dichloromethane was added and the mixture was washed with water. The solution was filtered through silica gel H(10.0 g), evaporated and the product crystallized from acetone–hexane. Compound **V** (350 mg) was obtained, m.p. 248–252°C; i.r.: 3420 and 3380 sh (O-H), 1652, 1615, and 875 cm^{-1} (4-en-3-one); NMR: $\delta(CDCl_3)$ 1.28 and 1.42 (s,s, acetonide-H_6), (s, 19-H_3), 3.60 and 3.67 (dd, $J = 13$ Hz, 21-h_2), 3.64 and 4.74 (dd, $J = 10$ Hz, 18-H_2), 4.52 (m, $W \simeq 8$ Hz, 11α-H), and 5.68 (s, 4-H): m.s.: The M^+ was not detected; prominent ions are at *m/z* 400 ($M^+ - 18$, loss of H_2O), *m/z* 387 ($M^+ - 31$, loss of CH_2OH), *m/z* 342 ($M^+ - 76$, loss of H_2O plus acetone): other prominent ions are at *m/z* 313, 299, 267, 249, 225, 207, 147, 117, 72. The base peak is at *m/z* 58 (acetone).

Synthesis of 18-*hydroxycortisol* (11β,17α,18,21-*tetrahydroxy*-4-*pregnene*-3,20-*dione*) (**VI**)

The acetonide (**V**) [300 mg] was dissolved in 50 ml tetrahydrofuran and 0.5 N HCl (10 ml) was added. After 1 h at room temperature the solution was extracted with dichloromethane. The dichloromethane was washed immediately with 5% sodium bicarbonate solution and water (2×). The solvent was evaporated and the crude material was purified by use of preparative TLC (silica gel plates, 2 mm thick) using a solvent mixture made of dichloromethane–methanol–triethylamine (92:8:0.1, by vol). 18-Hydroxycortisol **VI** was eluted with the solvent mixture dichloromethane-methanol–triethylamine (75:25:0.1, by vol). After evaporation, the residue was crystallized from acetone to yield 75 mg of product **VI**, m.p. 177–179°C; i.r.: 3440 (broad, O-H), 1655, 1615, and 870 (4-en-3-one), 1285, 1240, 1195, 1160, 1150, 1048(s) and 1010 cm^{-1}; NMR: $\delta(CDCl_3)$ 1.40 (s, 19-H_3), 3.675 and 3.885 (dd, $J = 12$ Hz, 21-H_2), 3.83 and 4.25 (dd, $J = 10$ Hz, 18-H_2), 4.00 *br s. OH*), 4.49 (t, $J \simeq 3$ Hz, 11α-H) and 5.64 (s, 4-H); $\delta(C_5D_5N)$ 1.59 (s, 19-H_3), 4.28 and 4.34 (dd, $J = 12$ Hz, 21-H_2), 4.26 and 5.07 (dd, $j = 10$ Hz, 18-H_2), 4.57 (m, $W \simeq 6$ Hz, 11α-H) 5.88 (s, 4-H): m.s.: the M^+ was not detected; prominent ions are at *m/z* 360 ($M^+ - 18$, loss of H_2O), *m/z* 347 ($M^+ - 31$, loss of CH_2OH), *m/z* 288, 270, 260, 228, 207, 189, 173, 147, 137, 123. 109, 97, 83, 60. The base peak is at *m/z* 163.

Synthesis of [1,2-^3H]18-*hydroxycortisol*-17α21-*acetonide*

18-Hydroxyprednisolone-17α,21-acetonide (30 mg) was sent to Amersham Corporation (Arlington Heights, IL) for catalytic tritiation with 5 Ci of carrier-free tritium gas in the presence of the homogeneous catalyst Tris(triphenylphosphine)rhodium (**I**) chloride. A total yield of 2.2 Ci of crude material was obtained (theoretical sp. act. 3 Ci/mmol). HPLC analysis of an aliquot showed it to be approx 90% pure. The crude material was purified in 100 mCi aliquots by HPLC using a 1 × 10 cm column filled with silica gel C-18 reverse phase material [10]. The column was eluted with methanol–acetonitrile (1:1, v/v) and water (60% by vol) containing 0.01% *N*-methyl-morpholine. The peak of radioactivity was pooled, evaporated under nitrogen at 40°C and stored at −60°C in toluene–isopropanol (9:1, v/v) containing 0.1% *N*-methyl-morpholine. Analytical HPLC demonstrated that the tritiated material was isopolar with authentic 18-hydroxycortisol-17α,21-acetonide (**V**). The tritiated material was further characterized by crystalization to constant specific activity. A mixture of 18-hydroxycortisol-17α,21-acetonide (50 mg) and [1,2 ^{3}H]18-hydroxycortisol-17α,21-acetonide (2.5 μCi) was crystalized 6 times from acetone or acetone–hexane. The specific activity of the last five mother liquors and final crystals were within 6% of the mean value.

Synthesis of [1,2 ^3H]18-*hydroxycortisol*

[1,2 ^{3}H]18-Hydroxycortisol-17α,21-acetonide (100 mCi) was dissolved in 2 ml of tetrahydrofuran and 0.2 ml of 1.0 N HCl were added. After 1 h triethylamine (0.1 ml) was added and the steroid extracted with 20 ml of ethyl acetate which was washed three times with water and evaporated. The steroid was purified by HPLC using a 1 × 25 cm column filled with polyhydroxylated silica gel (10 μ) material and eluted with toluene–isopropanol (94:6, v/v) containing 0.1% of *N*-methyl-morpholine. The peak of radioactivity containing [1,2 ^{3}H]18-hydroxycortisol was pooled and stored. Analytical HPLC of [1,2 ^{3}H]18-hydroxycortisol using a 5 Technosphere C-18 0.5 × 25 cm column and an elution system made of methanol–tetrahydrofuran–water (24.5:9.5:65, by vol) showed that the material was more than 98% pure and was isopolar with authentic unlabelled 18-hydroxycortisol.

Synthesis of 18-*hydroxycortisone* 17α,21-*acetonide* (17α,18,21-*trihydroxy*-4-*pregnene*-3,11,20-*trione*-17α,21-*acetonide* **(VII)**

18-Hydroxycortisol-17α,21-acetonide (150 mg) was dissolved in 25 ml of dichloromethane and pyridinium dichromate (150 mg) was added. The mixture was stirred for 1 h. TLC showed complete disappearance of the initial material. An additional 200 ml of dichloromethane was added and the mixture filtered and washed twice with water. Isopropanol was added to reach a concentration of 5% and the solution was filtered over 10 g of silica gel, evaporated and crystalized from acetone. The yield was 100 mg, m.p. 232–236°C; i.r.: ν_{max} 3500 (O-H), 1700 (11-CO), 1665 and 1620 cm^{-1} (4-en-3-one): NMR (C_5D_5N; 400 MHz) δ, 1.37, 1.39 (s,s, 19-H_3 and acetonide-H_3), 1.57 (s, acetonide-H_3), 3.57 and 3.92 (d, J = 9 Hz and dd, J = 9 and 0.9 Hz, respectively, 18-H_2), 3.94 and 4.03 (d,d, J = 11.5 Hz, 21-H_2), and 5.88 (s, 4-H).

Synthesis of 18-*hydroxycortisone* (17α,18,21-*trihydroxy*-4-*pregnene*-3,11,20-*trione*) **(VIII)**

Acetonide **VII** (75 mg) was dissolved in 5 ml of tetrahydrofuran and 1 ml of 1.0 N HCl was added. After 1 h TLC showed incomplete hydrolysis and a further 1 ml of 1.0 M HCl was added resulting in complete hydrolysis. After adding triethylamine to neutralize the HCl the material was extracted with 100 ml of ethyl acetate, washed with water twice, and the solvent was evaporated under vacuum at 40°C. The resulting powder was dissolved in hot ethyl acetate containing *N*-methyl-morpholine and crystalized. The yield was 40 mg. The steroid had a m.p. 214–219°C; i.r.: ν_{max} 3450–3280 (O-H), 1698 (11-CO), 1668 and 1622 cm^{-1} (4-en-3-one): NMR (C_5D_5N; 400 MHz): δ, 1.38 (s, 19-H_3), 3.02 (dd, $J_{12}\alpha, {}_{12}\beta$ = 12 Hz, $J_9\alpha, {}_{12}\alpha$ = 0.9 Hz, 12α-H), 3.15 (d, $J_{12}\alpha, {}_{12}\beta$ = 12 Hz, 12β-H) 3.99 and 4.06 (d,d, J = 9 Hz, 18-H_2), 4.23 and 4.31 (d,d, J = 12 Hz, 21-H_2), and 5.877 (d,d, J = 0.6 and 0.4 Hz, 4-H). The spectrum also showed the following signals due to *ca.* 10% of the minor isomer at C-20; δ, 1.42 (19-H_3), 4.01 and 4.105 (d,d, J = 9 Hz, 18-H_2), and 5.880 (s, 4-H).

DISCUSSION

Hydrogen abstraction by an alkoxyl radical requires an optimal distance of 2.5–2.7 Å from the alkoxyl radical to the carbon atom bearing the hydrogen to be abstracted [7]. In the case of saturated steroids almost exclusive attack at C-19 occurs. For steroids with a 4-en-3-one suction, a mixture of C-18 and C-19 functionalized products is formed. Extended conjugation (1,4 or 4,6 dienes, or a 1,4,6 triene) increases C-18 functionalization as a result of an increase of separation between C-19 and the 11β-oxygen atom [10]. It is for this reason and the relatively easy specific hydrogenation or tritiation with an homogeneous catalyst that we used the

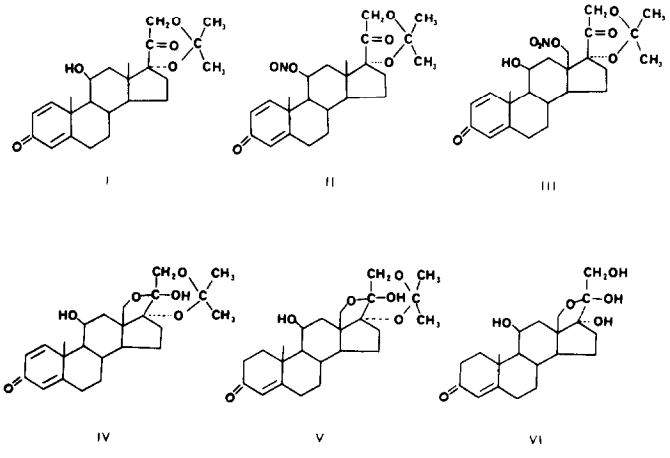
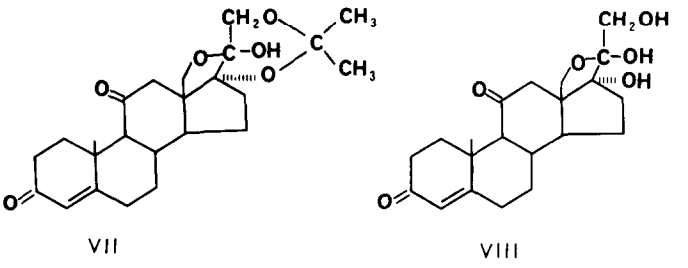


Fig. 1. Structure of steroid synthetic intermediates in the synthesis of 18-hydroxycortisol.



VII VIII

Fig. 2. Structure of steroid synthetic intermediates in the synthesis of 18-hydroxycortisone.

synthetic sequence leading to 18-hydroxycortisol prednisolone to start. Previous studies by Barton *et al.* [1] have shown that the dihydroxyacetone side chain of cortisol acetate or prednisolone acetate do not survive the irradiation procedure. We decided to use the 12α,21-acetonide instead because it can be hydrolyzed with dilute mineral acid under mild conditions. The popular bismethylenedioxy (BMD) system for protection of the side chain may also be an adequate derivative [11]. However, hydrolysis of BMD-steroids requires either harsh acid hydrolysis or refluxing in dilute acetic or formic acid which are conditions which could easily dehydrate 18-hydroxylated corticosteroids [1].

Pregnan-20-ones hydroxylated at C-18 occur mainly in the 18,20-hemiacetal form and, in our particular case, compounds **IV–VIII** also exist as 18,20 hemiacetals as shown by the absence of a C-20 carbonyl peak in the i.r. spectrum and from the relatively higher field position of the C-21 proton signal (δ3.6–3.7) in their NMR spectra compared with δ4.3 in the 20 oxo-compounds **I** and **II**. Many 18,20-hemiacetals have been observed to exist as mixtures of the (20*R*) and (20*S*) diastereoisomers by NMR spectrometry [12]. The proportions of the two forms vary according to other structural features of the molecule, but the form with C-21 in the "α" position and 20-OH "β", overhanging C-16, appears to predominate for steric reasons. The two forms often exhibit sufficient differences in their NMR spectra (particularly at high field) for some of the signals to be distinguished, although equilibration occurs sufficiently rapidly in solution to prevent separation.

Among the present compounds, 18-hydroxycortisone was found, from its NMR spectrum, to comprise a mixture (*ca* 10:1 respectively) of the (20*R*) and (20*S*) forms, and the NMR spectral details (at 400 MHz) are reported separately in the experimental section. The proportion of the (20*S*) form in 18-hydroxycortisol was distinctly smaller, although some of its weak NMR signals could be observed slightly shifted from those of the major (20*R*) isomer. The NMR spectrum of synthetic 18-hydroxycortisol (in $CDCl_3$) is identical to that reported for the natural compound [13]. The biological activity of 18-hydroxycortisol as a mineralocorticoid and glucocorticoid was found to be neglible [14].

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