Tetrahedron Vol. 38, No. 21, pp. 3173 to 3177, 1982
Printed in Great Britain.

0040-4020/82/213173-06$03.00/0

REDUCED METABOLITES OF 18-HYDROXY-11-DEOXYCORTICOSTERONE

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(*Received in the UK 5 February* 1982)

Abstract—Conditions are described for the preparation of the dihydro (**2** and **6**) and tetrahydro derivatives (**3**, **5** and **7**) of 18-hydroxy-11-deoxycortisterone (**1a**) by microbial transformations using *Clostridium paraputrificum* and chemical methods. The products were fully characterized and degraded into their respective 20→18 etiolactones. Microbial and chemical reductions of the Δ^4-3-ketolactone **8** are presented.

Recent studies on the metabolism of corticoid hormones suggest that their reduced metabolites are biologically active materials rather than merely catabolic products. It has been proven that several of the reduced derivatives of aldosterone are physiologically active *per se* and play a role in the regulation of aldosterone activity.[1,2] Furthermore, the 5α-dihydro derivative of 11-deoxycorticosterone has been shown to have a pronounced mineralocorticoid activity.[3-5] Melby *et al.*[6] have found that 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) (**1a**) (Scheme 1) is a major mineralocorticoid in the rat adrenal; it produces a significant rise in blood pressure without affecting the plasma potassium level.[7] With regard to sodium and potassium retention in dogs, 18-OH-DOC is 5- and 11-fold less potent than DOC, respectively. An excessive secretion of the tetrahydro derivative of 18-OH-DOC (18-OH-THDOC) has been reported in hypertensive patients with a supressed plasma renin activity.[8] Moreover, the same compound was found in the urine of patients with a 17-hydroxylase deficiency syndrome.[9]

The presence of the four 18-OH-THDOC derivatives has been shown in rat adrenal and liver extracts. However, these metabolites have not been actually isolated for the determination of their physical and biological properties.[10,11] Their presence in biological sources has only been ascertained by preparation of volatile methyloxime-trimethylsilyl (MO-TMS) derivatives, and comparison by gas chromatography-mass spectrometry with MO-TMS derivatives of a mixture of products obtained by reduction of 18-OH-DOC. The 3α,5β-isomer of 18-OH-THDOC (**3a**) has recently been chemically synthesized starting with 3α-acetoxy-5β-pregnane-20-one.[12]

Due to the increased interest in the biological properties of the reduced corticoids in general and the present need for obtaining them in amounts required for biological testing, suitable approaches for their synthesis had to be examined. In this communication we describe the preparation of the reduced derivatives of 18-OH-DOC, including their 20→18 etiolactones, by a combination of microbial bioconversions and chemical means.

During the past few years an ever-increasing use of the anaerobic microorganism *Clostridium paraputrificum* (Cp) for the reduction of the Δ^4-3-keto moiety in steroids has been reported.[13-17] One of the compounds quantitatively and specifically converted into the 3α,5β-tetrahydro derivative **3a** on a 1-mg scale was 18-OH-DOC (**1a**).[18] By substituting the mode of substrate addition from a methanol solution to a suspended neat powder and modifying the fermentation conditions we were able to prepare **3a** on a 100 mg scale in a 60% yield following a 72 h fermentation. While **3a** was the major product, a small amount of the 5β-dihydro compound **2a** was occasionally obtained. Surprisingly, in those experiments in which the acetate **1b** substituted the free alcohol **1a** as substrate (20–100 mg scale), the product isolated after 40 h fermentation was primarily the alcohol **2a** in 50–60% yield. Longer incubation times did not favor conversion to the tetrahydro stage. When pure **2a** was used as substrate most of the material was recovered unchanged: only small amounts of impure tetrahydro compounds were produced. Clearly the course of reduction in ring A with Cp is influenced by substrate concentration and by substituents in other sites of the molecule. Our observations are in agreement with recently described[17] reductions with Cp employing several other Δ^4-ketosteroid substrates in which, however, the differences between the various side-chains were much more pronounced than merely substitution of an alcohol by an acetate group. Of further interest is the observation that aldosterone could not be satisfactorily converted into the 3α,5β-tetrahydro derivative by this organism.[19]

In parallel with the microbial reduction of 18-OH-DOC a chemical route was also pursued. It had been previously found that the stepwise reduction in ring A of a variety of steroids is a dependable, if sometimes laborious, method of preparation of a number of metabolites. Thus catalytic reductions of cortisol and cortisone followed by further transformations furnished the 5α- and 5β-dihydro, 3α- and 3β-tetrahydro derivatives[20] and thence a variety of important 11-oxygenated metabolites.[21] In a similar fashion, the corresponding dihydro and tetrahydro metabolites were synthesized from corticosterone,[22] and the various analogous metabolites from compound S and DOCA.[23] Controlled hydrogenations of aldosterone and its derivatives also furnished the various di- and tetrahydro compounds.[24-26]

We have now hydrogenated 18-OH-DOC (**1a**) and the acetate **1b** in ethyl acetate containing some triethylamine in the presence of Pd–C, to furnish the 5β-isomers **2a** and **2b**, respectively. In addition, a small amount of the 5α-isomer was also present. In distinction, microhydrogenation in ethanol had previously yielded a 3:2 mixture of the 5α/5β-dihydro isomers.[10] The predominance of

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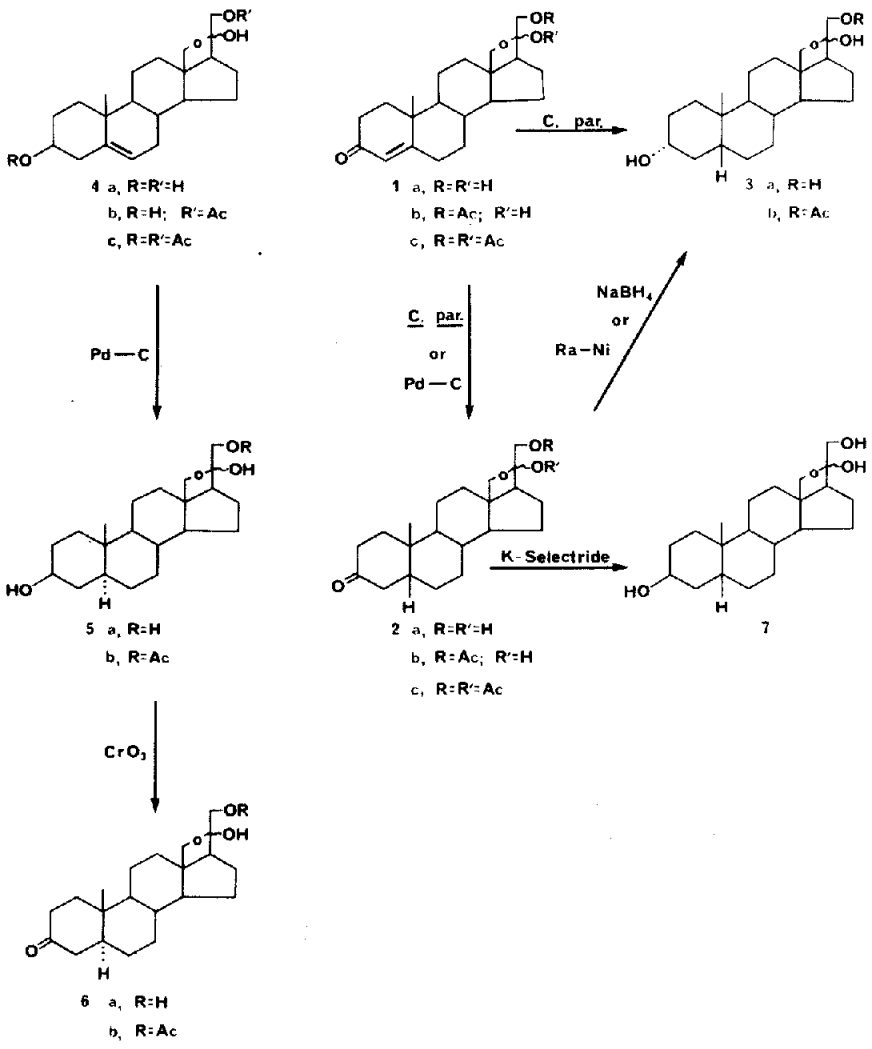

Scheme 1.

the 5β-isomer parallels the product distribution resulting from hydrogenation of DOCA with Pd,[23,27] the 18,20-hemiacetal structure apparently not having much influence on the ratio of isomers at position 5. The NMR spectrum of **2a** obtained from **1a** by the microbial reduction indicated a 1:1 ratio of isomers at carbon 20 (singlets at 3.68 and 3.76 ppm due to 18-CH_2), while in **2a** obtained by Pd hydrogenation of **1a** the corresponding signals signified a predominance of one of the isomers (ratio about 1:5; in **2b** obtained by hydrogenation the ratio was 1:3).

Sodium borohydride reduction of the 5β-isomer **2a** or **2b** furnished 18-OH-3α,5β-THDOC (**3a**), identical with an authentic sample.† Hydrogenation of the acetate **2b** with raney Ni yielded a mixture containing the acetate **3b**, the free triol **3a**, and a small amount of 18-OH-3β,5β-THDOC (**7**); hydrolysis of **3b** gave **3a**. A more convenient method for the preparation of **7** consisted of reduction of **2a** with K-Selectride®, a reagent known to reduce 3-ketocholestanes to their axial 3-ols.[28]

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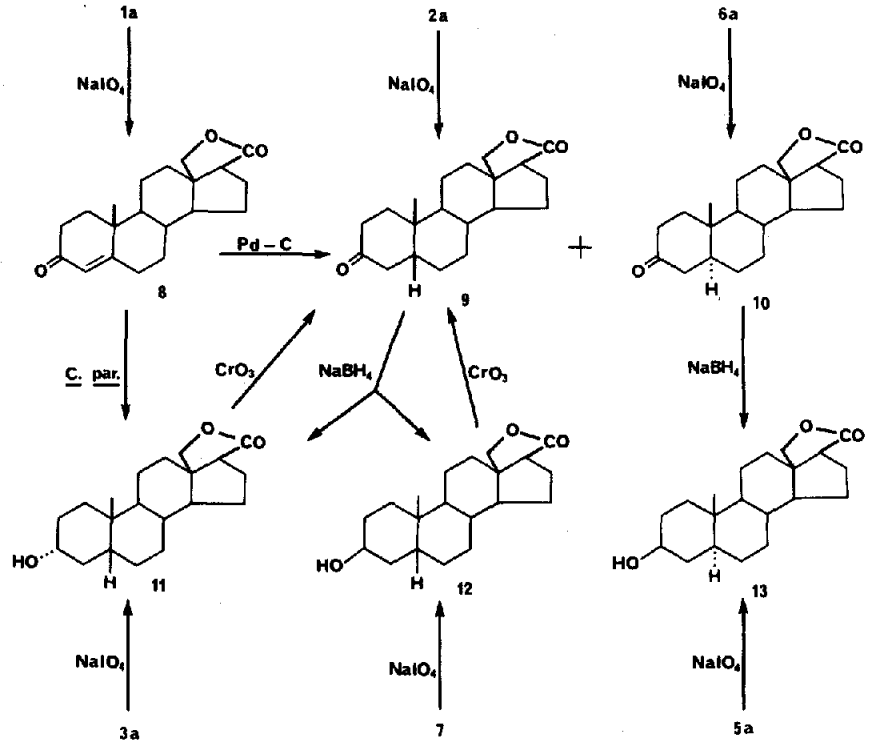
‡Kindly provided by Prof. R. Chayen, Endocrine Dept., Ichilov Hospital, Tel Aviv.

The 3β,5α-isomer **5a** was next prepared in a straightforward manner by hydrogenation of 18,21-dihydroxypregnenolone (**4a**)[29]. The product was identical with an authentic sample of **5a**,‡ and consisted of a *ca* 1:10 mixture of the two epimers at C-20. A similar hydrogenation of the 21-acetate **4b** furnished **5b**. Jones oxidation of the latter yielded 18-OH-5α-dihydro-DOC 21-acetate (**6b**) which was then hydrolyzed to the free triol **6a**.

Most of the dihydro and tetrahydro derivatives of 18-OH-DOC described above have rather wide mps and somewhat diffuse IR spectra, partly reflecting the presence of isomers at C 20, a situation which complicates purity determination. Furthermore, the dihydro compounds **2a** and **6a** have similar mobilities in TLC, as do compounds **3a** and **5a** (**7** has a slightly higher R_f value). It was hoped that the corresponding 20→18 lactones would be well-defined compounds with characteristic physical properties, in analogy with the aldosterone series.[24,30,31,32] For this purpose the etiolactone **8**[33,34] was submitted to microbial and chemical reductions and the products obtained were correlated with the various di- and tetra-hydro derivatives of 18-OH-DOC (Scheme 2). Incubation of **8** with Cp for 3 days in the usual nutrient medium caused complete con-



Scheme 2.

version to the 3α,5β-tetrahydrolactone **11**. When an aliquot was withdrawn after one day the product consisted of **11** and the starting material, but no dihydrolactone **9** was present. The latter compound could be isolated after 3 days' fermentation, in 10% yield only, if a relatively high concentration of substrate (80 mg/120 ml) was employed. The tetrahydrolactone **11** was identical with the product obtained by periodate oxidation of 18-OH-3α,5β-THDOC (**3a**).

Further correlation of the reduced 18-OH-DOC derivatives with the corresponding lactones was effected as follows: Jones oxidation of **11** furnished the 5β-dihydrolactone **9**, which, in turn, was found to be identical with the periodate oxidation product of 18-OH-5β-dihydro-DOC (**2a**). Sodium borohydride reduction of **9** gave **11** and the 3β,5β-lactone **12** in the ratio 3:1. The last compound was identical with the periodate oxidation product of 18-OH-3β,5β-THDOC (**7**). Jones oxidation of **12** gave **9**, confirming that **11** and **12** differ only with respect to configuration at position 3. Pd–C hydrogenation of the unsaturated lactone **8** furnished the two epimeric lactones **9** and **10** in roughly equal amounts. The latter was identical with the product of periodate oxidation of 18-OH-5α-dihydro-DOC (**6a**); sodium borohydride reduction of **10** yielded the 3β,5α-lactone **13**, which, in turn, could also be obtained by periodate oxidation of 18-OH-3β,5α-THDOC (**5a**).

As expected, all solid etiolactones obtained had sharp m.p.s and well-defined IR spectra, useful in identification of the parent 18-OH-DOC derivative.

EXPERIMENTAL

NMR spectra were obtained in $CDCl_3$ (TMS) on a Bruker WH-90 spectrometer. IR spectra were taken on a Perkin–Elmer 297 spectrophotometer in KBr pellets except where otherwise stated. Mass spectra were recorded with a DuPont 21-491B spectrometer. TLC spots were visualized by spraying with 10% H_2SO_4 in EtOH prior to heating.

18,21-*Dihydroxy-5β-pregnane-3,20-dione* 18,20-*hemiacetal* (18-OH-5β-*dihydro*-DOC)(**2a**)

(a) *By fermentation of* 18-OH-DOCA (**1b**) *with* Clostridium paraputrificum. A 200 ml narrow-mouth serum bottle was charged with 120 ml of a sterile nutrient soln containing 0.2% yeast extract, 0.5% Trypticase® soy broth, 0.8% glucose, 0.4% $NaHCO_3$, 0.1% NaCl, 0.1% $(NH_4)_2SO_4$, 0.02% $MgSO_4$, 0.01% $CaCl_2$, 0.0018% $FeSO_4 \cdot 7H_2O$, 0.05% K_2HPO_4, 0.063% KH_2PO_4, 0.1% cysteine and 0.0002% resazurin, adjusted to pH 7.0. A soln of 100 mg of **1b** in 1.5 ml MeOH was then injected through the septum under sterile conditions. The mixture was inoculated anaerobically with 5 ml of a starter culture of *Clostridium paraputrificum* ATCC 25780 (grown under the same conditions, without added steroids) and the resulting suspension was shaken in a gyratory shaker (250 rpm) at 37° for 40 h. The product was extracted with methylene dichloride (MDC), the emulsion was filtered through a celite pad and the aqueous phase was reextracted with two additional portions of the same solvent. The combined extracts were washed with $NaHCO_3$ aq, dried with Na_2SO_4 and evaporated. The residual solid was triturated with ether to yield **2a** (45 mg), m.p. 155–158°. Recrystallization from EtOAc containing a trace of Et_3N did not change the m.p.; λ_{max}^{KBr} 2.97 and 5.88 μ; δ 0.95 (s, 19-CH_3), 3.68 and 3.76 (ss, 18-CH_2, isomers at C-22, ratio 1:1), 3.66 and 3.82 (ABqs, J = 12, 21-CH_2) ppm; m/e 330 (M-18). The filtrates contained additional 10 mg of **2a** and several more polar components.

Acetylation of **2a** with Ac_2O–pyridine (1:1) overnight at room temp., followed by removal of solvents at 40° with a stream of N_2 and addition of water, furnished the 21-*acetate* **2b**, which after several recrystallizations from MDC–ether melted at 163–165°; λ_{max}^{KBr} 2.91 and 5.80 μ; *m/e* 372 (M-18), 330 (M-60).

(b) *By hydrogenation of* 18-OH-DOC (**1a**). A suspension of 250 mg of **1a** in 45 ml EtOAc was treated with 2 drops of Et_3N and 80 mg 5% Pd–C, and was stirred under H_2 for 2 h at room temp. The catalyst was filtered off and the soln concentrated to 10 ml; after 0.5 h the voluminous ppt consisting mostly of **2a** was collected, m.p. varying within the limits 127–131° to 142–143° in a

225 mg yield. The IR spectrum and TLC were identical with those of the product obtained above by fermentation, but the NMR spectrum was different: δ 0.96 (s, 19-CH_3), 3.65 and 3.76 (ss, 18-CH_2, isomers at C-20, ratio 1:5), 3.66 and 3.82 (ABqs, J = 11, 21-CH_2 in major isomer) ppm. The product contained a small amount of the 5α-epimer **6a**; after acetylation the product **2b** crystallized with some difficulty.

(c) *By hydrogenation of* 18-OH-DOCA (**1b**). Acetylation of **1a** with Ac_2O-pyridine (1:1) at room temp for several hr followed by quenching with ice–water gave **1b**[29,35,36] contaminated with a little **1c**,[37] distinguished on the TLC plate by the pink coloration of the faster-moving spot after developing with H_2SO_4. When 201 mg of this impure **1b** was recrystallized from ether, hydrogenated as described above for **1a** and the filtered soln evaporated, 68 mg of **2b** was obtained, m.p. 158–162.5° (ether); δ 0.96 (s, 19-CH_3), 2.13 (s, 21-OAc), 3.67 and 3.78 (ss, 18-CH_2, isomers at C-20, ratio 1:3), 4.03 and 4.14 (ABqs, J = 12, 21-CH_2 in minor isomer), 4.18 and 4.28 (ABqs, J = 11.5, 21-CH_2 in major isomer) ppm. The filtrate was chromatographed on a column of silica gel. Elution with 10% acetone in petroleum ether gave **2c** which separated from ether in clusters of prisms, m.p. 166–9.5°, 9 mg; λ_{max}^{KBr} 5.74 and 5.82 μ; δ 0.95 (s, 19-CH_3), 2.03 (s, 20-OAc), 2.13 (s, 21-OAc), 3.68 and 3.80 (ss, 18-CH_2, isomers at C-20, ratio 1:3), 4.00 and 4.18 (ABqs, J = 11.5, 21-CH_2 in minor isomer), 4.17 and 4.29 (ABqs, J = 11.5, 21-CH_2 in major isomer) ppm; m/e 372 (M-60), 312 (M-60-60). Further elution yielded **2b** which after recrystallization from ether melted at 138–145° (8 mg).

3α,18,21-*Trihydroxy-5β-pregnane*-20-*one* 18,20-*hemiacetal* (18-OH-3α,5β-THDOC) (**3a**)

(a) *By sodium borohydride reduction of* 18-OH-5β-*dihydro*-DOC (**2a**). A soln of 70 mg of **2a** of m.p. 153–156° (obtained by microbial reduction of **1b**) in 2 ml of THF was treated with 140 mg $NaBH_4$. Magnetic stirring was maintained for 5 min at 0°, then for 0.5 h at 10°. Water was added and the soln extracted with 3 × 20 ml portions of MDC. The extracts were dried with Na_2SO_4, treated with a drop of Et_3N and evaporated. The crystalline residue was washed with ether and had a m.p. of 143–155° (dec), 36 mg; the IR spectrum and TLC mobility were identical with those of an authentic sample of **3a**; *m/e* 332 (M-18). Two recrystallizations from EtOH with a drop of Et_3N furnished a sample melting at 181–182° (reported[12] 180–182°).

Instead of THF, MeOH could be used as solvent in the reduction; the acetate **2b** could also serve as the starting material. In some cases the product was accompanied by the more polar tetraol; column chromatography (silica gel, acetone–petroleum ether mixtures) was used to purify the material.

(b) *By raney nickel reduction of* 18-OH-5β-dihydro-DOCA (**2b**). A soln of 271 mg of **2b** in 30 ml dioxane was stirred for 4 h under H_2 at room temp and atmospheric pressure in the presence of 500 mg raney Ni. The filtered soln was evaporated and the oily residue chromatographed: elution with 20% acetone in petroleum ether furnished **3b** which crystallized from a small amount of EtOAc, m.p. 128–134°, 43 mg; λ_{max}^{KBr} 2.95 and 5.75 μ. The ester was difficult to purify and was best directly hydrolyzed as described below. Further elution with 20% acetone and then with 50% acetone in petroleum ether gave **3a**, which after crystallization from acetone containing 2 drops Et_3N had the m.p. 152–156° (57 mg), identical with the material obtained above. TLC of the filtrates showed the presence of a small amount of the 3β,5β-isomer **7**.

For *hydrolysis*, a soln of 38 mg of **3b** in 5 ml of 0.1 N methanolic KOH was refluxed for 0.5 h. The soln was evaporated to dryness *in vacuo* at room temp., the residual solid treated with 2 ml of water, the triol **3a** collected and washed with water. Recrystallization from acetone/Et_3N furnished 20 mg of pure **3a**, identical with the material isolated from the column.

(c) *By fermentation of* 18-OH-DOC (**1a**) *with Clostridium paraputrificum*. A sterile suspension of 95 mg of powdered **1a** in 120 ml of the nutrient soln (*vide supra*) was sealed and incubated with 5 ml of a pregrown culture of Cp. After shaking for 4 days at 37° the contents of 2 runs were pooled and worked up as described for the preparation of **2a**. The dried MDC soln containing a trace of Et_3N was concentrated to about 1 ml and 1 ml ether was added. The solid was collected and washed with ether to furnish 86 mg of **3a**, m.p. 154–167°. The IR spectrum and TLC were identical with those of a sample obtained under A. Column chromatography of the filtrate on silica gel using 20% acetone in petroleum ether furnished at first the 5β-dihydro compound **2a**, which after washing with ether weighed 14 mg, m.p. 152–156°, identical with the product obtained above. Further elution yielded additional 27 mg of **3a**, slightly contaminated with unreacted **1a**.

3β,18,21-*trihydroxy-5α-pregnane*-20-*one* 18,20-*hemiacetal* 21-*acetate* (**5b**). A warm soln of 171 mg of **4b**[29] in 50 ml EtOAc was cooled to room temp, treated with 5 drops Et_3N, 430 mg 5% Pd–C and hydrogenated for 4.5 h at room temp. The filtered soln was concentrated to 5 ml and allowed to stand overnight. The product was collected, m.p. 185–187°, having practically the same mobility as the starting material, but exhibiting a light brown coloration instead of pink with sulfuric acid spray. A further recrystallization raised the m.p. 192–193°; λ_{max}^{KBr} 2.90 and 5.72 μ; δ 0.75 (s, 19-CH_3), 2.12 (s, 21-OAc), 3.64 and 3.76 (ss, 18-CH_2, isomers at C-20, ratio 1:10), 4.00 and 4.18 (ABqs, J = 11.5, 21-CH_2 in minor isomer), 4.18 and 4.28 (ABqs, J = 11.5, 21-CH_2 in major isomer) ppm; *m/e* 374 (M-18), 332 (M-60), 314 (M-18-60).

3β,18,21-*trihydroxy-5α-pregnane*-20-*one* 18,20-*hemiacetal* (18-OH-3β,5α-THDOC) (**5a**). The crude **4a** (60 mg, about 90% pure), obtained by saponification of **4c**, was hydrogenated as described for **4b**. The product which separated from the concentrated EtOAc soln was recrystallized from acetone containing 2 drops of Et_3N, and melted at 183–184° (15 mg). The IR spectrum and chromatographic mobility were identical with those of an authentic sample of **5a**; δ 0.75 (s, 19-CH_3), 3.62 and 3.73 (ss, 18-CH_2, isomers at C-20, ratio 1:10), 3.18 and 3.65 (ABqs, J = 11.5, 21-CH_2 in major isomer), 3.60 (m, 3α-H) ppm.

18,21-*dihydroxy-5α-pregnane*-3,20-*dione* 18,20-*hemiacetal* 21-*acetate* (**6b**). A soln of 85 mg of the 3β-ol **5b** in 15 ml acetone was concentrated to 10 ml, cooled in ice, and treated with 0.2 ml of the Jones reagent. After 5 min at 0° 10 ml of a 5% Na_2SO_3 aq was added and the mixture was concentrated *in vacuo* at room temp. to remove the bulk of acetone. The crystalline material was collected, washed with water and taken up in MDC. The green impurity was filtered off, the solvent evaporated and the product crystallized from ether to furnish 31 mg of **6b**, m.p. 154–155°. Recrystallization from EtOAc containing a drop of Et_3N gave 7 mg, m.p. 158–161°; λ_{max}^{KBr} 2.87, 5.79 and 5.87 μ; δ 0.95 (s, 19-CH_3), 2.13 (s, 21-OAc), 3.67 and 3.78 (ss, 18-CH_2, isomers at C-20, ratio 1:10), 4.17 and 4.29 (ABqs, J = 11.5, 21-CH_2 in major isomer) ppm; *m/e* 372 (M-18), 330 (M-60).

18,21-*dihydroxy-5α-pregnane*-3,20-*dione* 18,20-*hemiacetal* (18-OH-5α-*dihydro*-DOC) (**6a**). A soln of 30 mg of **6b** in 5 ml of 0.1 N methanolic KOH was refluxed for 0.5 h. The solvent was evaporated *in vacuo* at room temp., 2 ml water was added, the crystals were collected and washed with water (20 mg).

The recrystallized product (acetone with a trace of Et_3N) had the m.p. 178–180°; λ_{max}^{KBr} 2.94 and 5.88 μ; δ 0.95 (s, 19-CH_3), 3.68 and 3.76 (ss, 18-CH_2, isomers at C-20, ratio 1:10), 3.62 and 3.80 (ABqs, J = 11.5, 21-CH_2 in minor isomer), 3.64 and 3.80 (ABqs, J = 11.5, 21-CH_2 in major isomer) ppm.

3β,18,21-*trihydroxy-5β-pregnane*-20-*one* 18,20-*hemiacetal* (18-OH-3β,5β-THDOC) (**7**). A soln of 200 mg of **2b** (slightly contaminated with the diacetate **2c**) in 5 ml dry THF was treated with 2 ml of 1 M K-Selectride® (potassium tri-sec-butylborohydride) from a syringe in a N_2 atmosphere with magnetic stirring and ice-cooling. The yellow soln was stirred for an additional 2 h at 0° and then for a further 2 h at 10°. The mixture was next cautiously treated at 0° with 0.8 ml of 10% NaOHaq, followed by 0.8 ml of 30% H_2O_2. After 10 min the mixture was worked up with water and MDC. Evaporation of the organic solvent containing a trace of Et_3N furnished a gum which was chromatographed on silica gel. Elution with 20% and 50% acetone in petroleum ether yielded an ester of unknown structure (55 mg, m.p. 125–131°), followed by 65 mg of the expected 3β,5β-isomer **7**, m.p. 117–121°. Two recrystallizations from EtOAc with a drop of Et_3N raised the m.p. to 133–135°; λ_{max}^{KBr} 3.00 μ; δ 0.90 (s, 19-CH_3), 3.62 and 3.73 (ss, 18-CH_2, isomers at C-20, ratio 1:10), 3.29 and 3.66 (ABqs J = 10.5, 21-CH_2 in major isomer), 3.73 (m, 3α-H) ppm; *m/e* 332 (M-18).

A similar reduction of **2a** (170 mg, suspended in 5 ml of dry THF) provided a crude material which by chromatography or direct crystallization from EtOAc with a trace of Et_3N gave a solid, m.p. 166–170° (20 mg), devoid of the 19-CH_3 singlet. The filtrate gradu-

ally crystallized and must have contained the desired isomer **7** because periodate oxidation afforded mostly the corresponding lactone **12**.

18-hydroxy-4-androstene-3-one-17β-carboxylic acid lactone (20→18) (**8**) was obtained from 18-OH-DOC (**1a**) essentially as described[33] except that sodium periodate was used instead of periodic acid. From 600 mg of **1a** 504 mg of water-washed and air-dried material was obtained, m.p. 227–229° (reported 221–224°,[33] 227–231°,[34] 228–232°,[38] 222–226°,[39]) and subsequently used as such.

18-hydroxy-5α-androstane-3-one-17β-carboxylic acid lactone (20→18) (**10**). Similarly, 7 mg of **6a** afforded 4 mg of the lactone **10**, m.p. 230–234°, identical to a sample described below.

18-hydroxy-5β-androstane-3-one-17β-carboxylic acid lactone (20→18) (**9**). In a similar manner, 20 mg of **2a** furnished 12 mg of **9**, m.p. 175–182°, whose properties were identical with those of a sample obtained below.

3β,18-dihydroxy-5β-androstane-17β-carboxylic acid lactone (20→18) (**12**). In an analogous manner 21 mg of **7** was transformed into **12**, 17 mg, m.p. 155–180°. Recrystalization from EtOAC—petroleum ether gave a sample, m.p. 185–187°, identical with the product obtained below.

3α,18-dihydroxy-5β-androstane-17β-carboxylic acid lactone (20→18) (**11**). Periodate oxidation of **3a** furnished **11** as a gum which resisted all attempts at crystallization and whose spectral properties and chromatographic mobility were identical with those of the sample described below.

3β,18-dihydroxy-5α-androstane-17β-carboxylic acid lactone (20→18) (**13**). In a similar fashion 8.5 mg of **5a** gave 6 mg of **13**, m.p. 210–9°. Recrystallization from EtOAc gave a product of m.p. 220–222°, identical with a sample synthesized as described below.

Hydrogenation of 18-hydroxy-4-androstene-3-one-17β-carboxylic acid lactone (20→18) (**8**). A suspension of 162 mg of **8** in 20 ml EtOAc containing 2 drops Et_3N was stirred in a H_2 atmosphere for 2 h at room temp. in the presence of 70 mg 5% Pd-C. Evaporation of the filtered soln and fractional crystallizations of the solid residue from acetone furnished a total of 76 mg of the 5α-isomer **10**, a sample of which melted at 230–235° (reported 239°[40]); λ_{max}^{KBr} 5.68 and 5.88 μ; δ 0.97 (s, 19-CH_3, 4.01 and 4.07 (ABq, J = 10, 18-CH_2) ppm; *m/e* 316.

Crystallization of the filtrates from acetone–ether deposited a total of 82 mg of the 5β-epimer **9** of m.p. 163–173°. The pure sample had the m.p. 186–187° (MDC–petroleum ether); λ_{max}^{KBr} 5.68 and 5.88 μ; δ 0.98 (s, 19-CH_3), 4.01 and 4.07 (ABq, J = 10, 18-CH_2) ppm; *m/e* 316.

Sodium borohydride reduction of 18-hydroxy-5α-androstane-3-one-17β-carboxylic acid lactone (20→18) (**10**). A warm soln of 79 mg of **10** in 10 ml MeOH was treated with 2 ml 10% NaOHaq, followed by 200 mg $NaBH_4$. After allowing to stand at room temp. for 2 h most of the solvents were removed *in vacuo*, 5 ml of water was added and the mixture cautiously acidified with HCl at 0°. The solid was collected and washed with water to afford 68 mg of **13**, m.p. 194–212°. Two recrystallizations from EtOAc raised the m.p. to 220–222°; λ_{max}^{KBr} 2.90 and 5.61 μ; δ 0.76 (s, 19-CH_3), 3.98 and 4.05 (ABq, J = 10, 18-CH_2), 3.59 (m, 3α-H) ppm; *m/e* 318, 300 (M-18).

Sodium borohydride reduction of 18-hydroxy-5β-androstane-3-one-17β-carboxylic acid lactone (20→18) (**9**) was carried out as described for **10**. Starting with 91 mg of material the oily product was taken up in MDC and chromatographed on silica. Elution with 10% and 20% acetone in petroleum ether furnished several crystalline solids. The first was the 3β,5β-isomer **12** (10 mg), m.p. 178–180° (ethyl acetate–petroleum ether); λ_{max}^{KBr} 2.94 and 5.66 μ; δ 0.92 (s, 19-CH_3), 3.98 and 4.04 (ABq, J = 10, 18-CH_2), 4.15 (m, 3α-H) ppm; *m/e* 300 (M-18). Further elution yielded the 3α,5β-isomer **11** as a gum, 30 mg; λ_{max}^{film} 2.93 and 5.68 μ; δ 0.88 (s, 19-CH_3), 3.97 and 4.03 (ABq, J = 10, 18-CH_2, 3.65 (m, 3β-H) ppm; m/e 300 (M-18). Other accompanying materials included apparent products of reduction of the lactone ring.

Fermentation of 18-hydroxy-4-androstene-3-one-17β-carboxylic acid lactone (20→18) (**8**) *with* Clostridium paraputrificum. A soln of 80 mg of **8** in 2 ml warm MeOH was injected under sterile conditions into a 200-ml bottle containing 100 ml of a nutrient soln, as described for the conversion of **1b** into **2a**. The bottle was inoculated with 7 ml of a growing culture of Cp and the suspension was shaken for 3 days. The contents of 3 bottles were pooled and worked up as above; the oily residue had no absorption in the UV. Chromatography on silica gel (elution with 20% acetone in petroleum ether) furnished the 5β-dihydrolactone **9**, which after recrystallization from MDC–petroleum ether melted at 186–187° (20 mg) and was identical with the sample obtained above. Further elution afforded 160 mg of the pure gummy 3α,5β-tetrahydrolactone **11**, identical with the material obtained above.

In another series of experiments (concentration of substrate 50 mg per 120 ml of nutrient soln) an aliquot withdrawn after 1 day showed the presence of **11** and the starting material **8**, but no dihydrolactone; after a total of 3 days, only the tetrahydrolactone **11** was present.

Oxidation of **12** *into* **9**. A soln of 8 mg of **12** in 2 ml acetone was treated at 0° with 3 drops of the Jones reagent. After 5 min 2 drops MeOH was added followed by 1 ml of water, and the mixture was concentrated *in vacuo* at room temp. almost to dryness. The solid was collected and washed with water to furnish 5 mg, m.p. 179–184°, identical with **9** obtained above.

Oxidation of **11** *into* **9**. An analogous conversion of **11** (24 mg) furnished **9** (16 mg), m.p. 183–185.5°, identical with the above material.

Note added in proof. **6b** has now been converted with K-Selectride into 3α,18, 21 - trihydroxy - 5α - pregnane - 20 - one, 18,20 - hemiacetal (18 - OH - 3α,5α - THDOC), m.p. 159–162° (EtOAc); λ_{max}^{KBr} 2.98 μ; δ 0.72 (s, 19-CH_3), 3.73 (s, 18-CH_2), 3.22 and 3.64 (ABqs, J = 9.6, 21-CH_2), 4.04 (m, 3β-H) ppm; m/e 332 (M-18), 302 (M-36). Periodate oxidation of the latter furnished 3α,18 - dihydroxy - 5α - pregnane - 17β - carboxylic acid lactone (20→18), m.p. 151–152° (MDC-petroleum ether); λ_{max}^{KBr} 2.92 and 5.64 μ; δ 0.73 (s, 19-CH_3), 3.98 and 4.05 (ABq, J = 9.6, 18-CH_2), 4.04 (m, 3β-H) ppm; m/e 318, 300 (M-18).

Acknowledgement—The authors wish to thank Mrs. Sharon Linder for excellent secretarial work, Mrs. Sara Ben-Chayim for the NMR measurements and Mr. Shimon Hauptmann for recording the mass spectra.

REFERENCES

[1]D. J. Morris and R. P. Davis, *Metabolism* **23**, 473 (1974).
[2]D. J. Morris, *Endocrine Reviews* **2**, 234 (1981).
[3]B. E. Michael, M. H. Holbrook, S. L. Dale and J. C. Melby, *61st Ann. Meeting of the Endocrine Soc.* abstracts, p. 235, 13–15 June 1979.
[4]H. Sekihara and D. P. Island, *60th Ann. Meeting of the Endocrine Soc.*, abstracts, p. 399, 14–16 June (1978).
[5]J. Carroll, P. Komanicky and J. C. Melby, *Steroids* **37**, 111 (1981).
[6]J. C. Melby, S. L. Dale, R. J. Grekin, R. Gaunt and T. E. Wilson, *Recent Prog. Horm. Res.* **28**, 287 (1972).
[7]J. Carroll, P. Komanicky and J. C. Melby, *J. Steroid Biochem.* **14**, 989 (1981).
[8]G. Constantopoulos, M. Kusomoto, J. Gutkowska and J. Genest, *Can. J. Physiol. Pharmacol.* **57**, 1407 (1979).
[9]J. W. Honour, J. Tourniaire, E. G. Biglieri and C. H. L. Shackleton, *J. Steroid Biochem.* **9**, 495 (1978).
[10]M. Prost, P. Bournot and B. F. Maume, *Steroids* **25**, 177 (1975).
[11]P. Bournot, M. Prost and B. F. Maume, *J. Chromat.* **112**, 617 (1975).
[12]M. Hossain and D. N. Kirk, *Steroids* **34**, 677 (1979).
[13]V. D. Bokkenheuser, J. B. Suzuki, S. B. Polovsky, J. Winter and W. G. Kelly, *Applied Microbiol.* **30**, 82 (1975).
[14]V. D. Bokkenheuser, J. Winter and P. Dehazya, O. de Leon and W. G. Kelly, *J. Steroid Biochem.* **7**, 837 (1976).
[15]J. Winter, V. D. Bokkenheuser and L. Ponticorvo, *J. Biol. Chem.* **254**, 2626 (1979).
[16]T. J. Glass, L. A. Wheeler, V. L. Sutter and S. M. Finegold, *Biochim. Biophys. Acta* **573**, 332 (1979).
[17]A. Fauve and A. Kergomard, *Tetrahedron* **37**, 899 (1981).
[18]C. H. L. Shackleton, J. W. Honour, J. Winter and V. D. Bokkenheuser, *J. Steroid Biochem.* **11**, 1141 (1979).
[19]D. N. Kirk and B. W. Miller, *J. Chem. Soc.* Perkin I, 2818 (1980).

[20]M. Harnik, *J. Org. Chem.* **28**, 3386 (1963).
[21]M. Harnik, *Israel J. Chem.* **1**, 158 (1963).
[22]M. Harnik, *Steroids* **2**, 485 (1963).
[23]M. Harnik, *Ibid* **3**, 359 (1964).
[24]H. Kohler, R. H. Hesse and M. M. Pechet, *J. Biol. Chem.* **239**, 4117 (1964).
[25]Y. Lederman, R. Szpigielman, M. Bendcovsky, J. Herling and M. Harnik, *Anal. Biochem.* **51**, 193 (1973).
[26]M. Harnik, Y. Lederman, R. Szpigielman and J. Herling, *Tetrahedron* **32**, 1001 (1976).
[27]A. Wettstein and F. Hunziker, *Helv. Chim. Acta* **23**, 764 (1940).
[28]R. Contreras and L. Mendoza, *Steroids* **34**, 121 (1979).
[29]D. N. Kirk and M. S. Rajagopalan, *J. Chem. Soc.* Perkin I, 1860 (1975).
[30]W. G. Kelly, L. Bandi and S. Lieberman, *Biochemistry* **1**, 792 (1962).
[31]S. Ulick and K. K. Vetter, *J. Biol. Chem.* **237**, 3364 (1962).
[32]S. Ulick, H. C. Rose and L. C. Ramirez, *Steroids* **16**, 183 (1970).
[33]F. W. Kahnt, R. Neher and A. Wettstein, *Helv. Chim. Acta* **38**, 1237 (1955).
[34]R. Pappo, *J. Am. Chem. Soc.* **81**, 1010 (1959).
[35]D. H. R. Barton, M. J. Day, R. H. Hesse and M. M. Pechet, *J. Chem. Soc.* Perkin I, 2252 (1975).
[36]M. Biollaz, J. Kalvoda and J. Schmidlin, *Helv. Chim. Acta* **58**, 1425 (1975).
[37]A. K. Roy, L. C. Ramirez and S. Ulick, *J. Steroid Biochem.* **7**, 81 (1976).
[38]H. Levy, D. I. Cargill and C. H. Cha, *Steroids* **5**, 131 (1965).
[39]J. Hora and V. Cerny, *Coll. Czech. Chem. Comm.* **30**, 1169 (1965).
[40]A. Kasal, V. Cerny and F. Sorm, *Ibid* **28**, 411 (1963).