SYNTHESIS AND STEREOCHEMISTRY OF 7β- AND 7α-AMINO-, ACETAMIDO-, HEMISUCCINAMIDO- AND TEREPHTHALAMIDO DERIVATIVES OF TESTOSTERONE

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Received 2-12-81

ABSTRACT

The reduction of 3-ethylenedioxy-7-oximino-5-androsten-17β-yl acetate and of its 17β-tetrahydropyranyl ether analog with sodium in ethanol, followed by thin-layer chromatography, allowed the isolation of the corresponding 17β-hydroxy- and 17β-tetrahydropyranyloxy-5-en-7β- and 7α-amines which were also characterized as 7-acetamides. The acylation of the two epimeric 17β-hydroxy-5-en-7-amines with succinic anhydride followed by selective saponification of the 17β-hemisuccinate group and diazomethane esterification, gave the corresponding 17β-hydroxy-5-en-7β- and 7α-hemisuccinamido methyl esters characterized also as 17β-acetates. On the other hand, the acylation of the two 17β-tetrahydropyranyloxy-5-en-7-amines with the acid chloride of terephthalic acid monomethyl ester led to the more rigid 7β- and 7α-terephthalamido methyl ester side-chains. The acidolysis of the 3-ethyleneketal protecting group of the preceding 5-en-7-N-acyl derivatives regenerated the 4-en-3-oxo function while the 17β-tetrahydropyranyl ether group was cleaved simultaneously into the 17β-alcohol. The four desired 7β- and 7α-hemisuccinamido- and terephthalamido carboxylic side-chain derivatives of 17β-hydroxy-4-androsten-3-one (testosterone) were finally obtained by saponification of the corresponding methyl esters.

INTRODUCTION

The introduction of a reactive side-chain in a position different from those bearing the characteristic functional groups of a steroid hormone, followed by covalent coupling of this side-chain to an immunogenic protein carrier still remains the prerequisite step of most of the recent attempts aimed at obtaining anti-steroid antisera of improved specificity [1].

Unfortunately, despite numerous attempts [2-5], C-7 linked steroids have failed to elicit antibodies specific enough to distinguish perfectly between the two 4-en-3-oxo- and 5α-H-3-oxo structures. Nevertheless, it has been found that in some cases this cross-reaction was significantly decreased after fractionation of anti-7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one antisera on appropriate 17β-hydroxy-5α-androstane-linked immunoadsorbents [6].

With a view to developing the above fractionation experiments, the present paper describes the introduction of the epimeric 7β- and 7α-amines on the 17β-

hydroxy-4-androsten-3-one molecule (testosterone) by reduction of 3-ethylenedioxy-5-en-7-oximino precursors with sodium in ethanol [7, 8] and the subsequent acylation of the resulting 3-ethylenedioxy-5-en-7β- and 7α-amines into the corresponding acetamido-, hemisuccinamido- and terephthalamido derivatives which were finally converted into the desired 4-en-3-oxo-7-derivatives by acidolysis of the 3-ethyleneketal protecting group.

However, the 7-hemisuccinamido carboxylic side-chains might show a poor ability to hold the steroid apart from the protein carrier, owing to possible folded conformations stabilized by intramolecular hydrogen bonding between the 7-amide and the terminal amide group formed after peptidic coupling to the ε-amine of the lysine residues of the protein. Therefore, more rigid 7-terephthalamido carboxylic side-chains, as opposed to the 7-hemisuccinamido side-chains, were also fixed for the purpose of increasing the distance between the steroid and the carrier protein or the affinity matrix, thus allowing a better accessibility of the steroid molecule for immunological recognition or affinity fractionations.

Furthermore, the stability of the 7-amide linkage in strongly acidic or basic media [8], as well as the ease with which different side-chains can be coupled to 7-amino precursors are potential advantages for further biological use.

SYNTHESIS

I. PREPARATION OF THE 7-AMINO- AND 7-ACETAMIDO DERIVATIVES

For the sake of clarity, the spectrometric evidence supporting the 7β- and 7α-configurations mentioned below is presented at the end of this paper (see IV).

1°) Ethylenedioxy-5-en-7β- and 7α-amines and acetamides (Fig. 1):

The 3-ethylenedioxy-7-oximino-5-androsten-17β-yl acetate (2) was prepared by condensation of hydroxylamine hydrochloride with the 5-en-7-ketone (1) in pyridine solution, at room temperature [9]. The reduction of this conjugated oxime with sodium in ethanol [7] gave a mixture of 3-ethylenedioxy-5-en-7-amines and of their 5,6-dihydro analogs which was separated by thin-layer chromatography (t.l.c.) on silica gel plates ($CHCl_3$-MeOH-NH_4OH 100:10:1). The faster-moving compound (26 %) was identified as the 5-en-7β-amine (3) characterized also as the 5-en-7β-acetamide (7). The intermediate spot (30 %) was found to contain an inseparable mixture of 5α-H-7β- and 7α-amines, (5) and (6), characterized as 7β- and 7α-acetamides, (9) and (10) [3]. The slower-moving compound (17 %), identified as the 5-en-7α-amine (4), was also characterized as the corresponding 5-en-7α-acetamide (8). Preparative t.l.c. on silica gel (EtOAc) of the acetylated mixture of crude 7-amines allowed the separation of the slower-

moving 5-en-7α-acetamide (8) from the intermediate 5α-H-7β-acetamide (9), but not that of the 5-en-7β-acetamide (7) from the 5α-H-7α-acetamide (10). However, the use of $CHCl_3$-MeOH mixtures resulted in an opposite order of polarities, the 5α-dihydro-7β- and 7α-contaminants becoming superimposed on the spots of the 5-en-7β- and 7α-acetamides, respectively (slower-moving 7β-epimer).

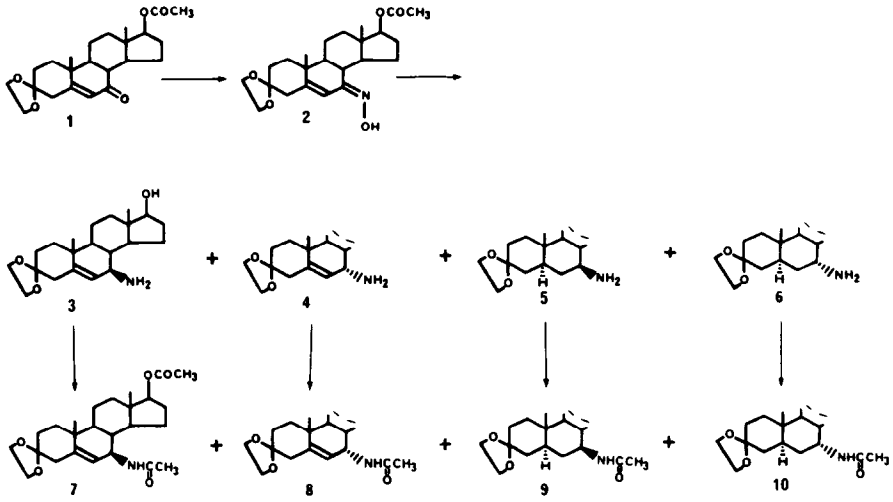


Fig. 1

2°) 4-En-3-oxo-7β- and 7α-amines and acetamides (Fig. 2) :

The acidolysis (HCl-dioxane) of the 3-ethyleneketal group of the pure 5-en-7β- and 7α-amines, (3) and (4), gave the 4-en-3-oxo-7β- and 7α-amines, (11) and (12) respectively. The acidolysis of 3-ethylenedioxy-5-en-7β- or 7α-amines partially contaminated with 5,6-dihydro by-products led to a mixture of the corresponding 4-en-3-oxo-7-amines (one single faster-moving spot after t.l.c. on silica gel, $CHCl_3$-MeOH-NH_4OH 100:10:1) and of the previously described 3-oxo-5α-H-7β- and 7α-amines [3] which were both found in the same slower-moving spot. Preparative t.l.c. always led to the formation of 5-10% of 4,6-dien-3-ketone [9–11], thus requiring a final purification of 4-en-3-oxo-7-amines as hydrochlorides.

The 4-en-3-oxo-7β- and 7α-amines, (11) and (12), were characterized as 7β- and 7α-acetamides, (13) and (14), which were also obtained in nearly quantitative yields after acidolysis (HCl-dioxane) of the two 3-ethylenedioxy-5-en-7β- and 7α-acetamides, (7) and (8). As expected from the stability of the 7-acetate analogs [11], no 4,6-dien-3-oxo by-product was formed. This acidolysis step was followed by t.l.c. on silica gel in order to prevent the acid cleavage of the 17β-acetate

group. The use of petroleum ether-EtOAc or $CHCl_3$-EtOAc mixtures was found to lead to an inversion of the Rf values of the 4-en-3-oxo-7β- and 7α-acetamides (slower-moving 7β-epimer), as compared to those of their corresponding 3-ethylenedioxy-5-ene precursors (slower-moving 7α-epimer), whereas the acidolysis of the 3-ethylenedioxy-5α-H-7β- and 7α-acetamides, (9) and (10), into the known 3-oxo-5α-H-7β- and 7α-acetamides [3] did not change the order of polarities (slower-moving 7β-epimer). This change of polarities facilitated the direct purification of the slower-moving 4-en-3-oxo-7β-acetamide from 5,6-dihydro contaminants by t.l.c. of the acetylated mixture of crude 7-amines. However, the above inversion of the Rf values did not occur with $CHCl_3$-MeOH mixtures.

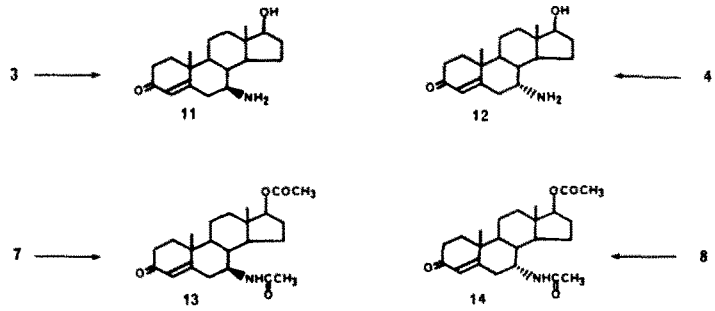


Fig.2

II. SYNTHESIS OF THE 7-HEMISUCCINAMIDO DERIVATIVES

1°) 3-Ethylenedioxy-5-en-7β- and 7α-hemisuccinamido methyl esters (Fig. 3) :

The two pure 3-ethylenedioxy-5-en-7β- and 7α-amines, (3) and (4), were treated with an excess of succinic anhydride in pyridine solution and gave the corresponding 7,17-dihemisuccinoylated products. The 17-hemisuccinate group was selectively saponified [8] after refluxing the crude 7,17-dihemisuccinoylated products overnight in a methanolic solution of potassium hydroxide, thus giving the 3-ethylenedioxy-5-en-17β-hydroxy-7β- and 7α-hemisuccinamides. These carboxylic acids were esterified with an ethereal solution of diazomethane to the 17β-hydroxy-7β- and 7α-hemisuccinamido methyl esters, (15) and (16) respectively, characterized also as the corresponding 17β-acetoxy-7β- and 7α-hemisuccinamido methyl esters, (17) and (18).

A similar treatment of the crude mixture of 7-amines followed by t.l.c., allowed the isolation of the 5-en-7α-hemisuccinamido methyl ester (18) from the 5α-dihydro contaminants, (19) and (20), but not that of the 5-en-7β-epimer (17).

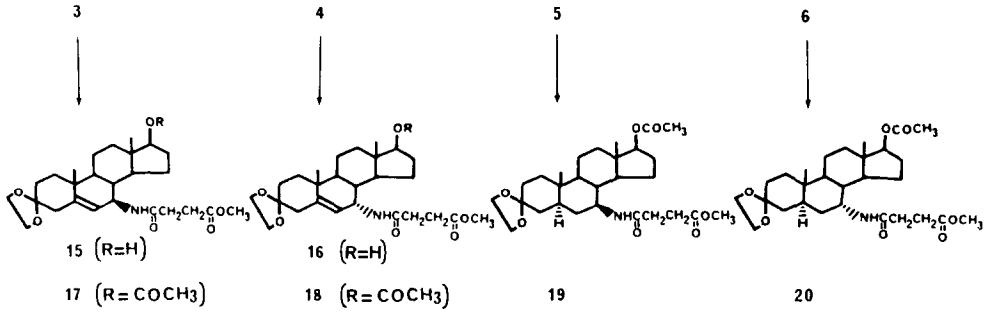


Fig.3

2°) 4-En-3-oxo-7β- and 7α-hemisuccinamides and methyl esters (Fig. 4) :

The two pure 17β-hydroxy-4-en-3-oxo-7β- and 7α-hemisuccinamido methyl esters, (21) and (22), as well as their 17β-acetoxy analogs, (23) and (24), were obtained in nearly quantitative yields after acidolysis (HCl-dioxane) of each of the corresponding 3-ethylenedioxy-5-en-7β- and 7α-hemisuccinamido methyl ester precursors, (15) and (16) or (17) and (18).

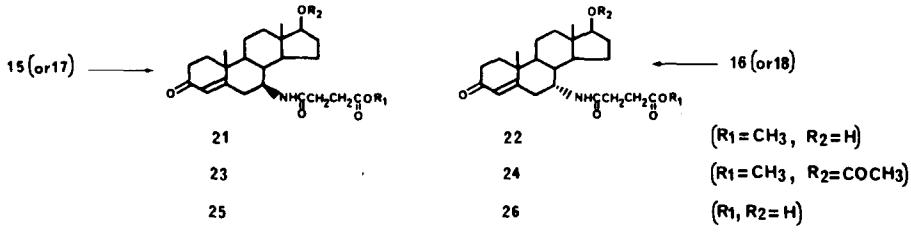


Fig.4

As mentioned above in the case of the 7-acetamido analogs, the acidolysis of the 3-ethylenedioxy-5-en-7-hemisuccinamido methyl esters was found to lead to an inversion of the order of the Rf values of the 4-en-3-oxo-7β-and 7α-epimers but not of the 3-oxo-5α-H-7β- and 7α-hemisuccinamido methyl esters [31], when t.l.c. was performed on silica gel with petroleum ether-EtOAc or $CHCl_3$-EtOAc mixtures. This inversion of polarities facilitated the direct isolation of the slower-moving 4-en-3-oxo-7β-epimers from the mixture of 7-hemisuccinamido methyl esters obtained directly from the crude mixture of 7-amines.

The two desired 17β-hydroxy-4-en-3-oxo-7β- and 7α-hemisuccinamides, (25) and (26), were obtained in nearly quantitative yields either from the corresponding 17β-hydroxy-7β- and 7α-hemisuccinamido methyl esters, (21) and (22), or from their 17β-acetoxy analogs, (23) and (24), after saponification with potassium

hydroxide in ethanol followed by acid precipitation of the free hemisuccinamides. These two carboxylic acids were distinguishable by t.l.c. on silica gel ($CHCl_3$-acetone-acetic acid 7:2:1, slower-moving 7β-epimer). The structures of these carboxylic acids were unambiguously confirmed by diazomethane esterification in $CHCl_3$ [31], followed by acetylation, which gave successively, quantitative yields of the already characterized 17β-hydroxy-4-en-3-oxo-7β- and 7α-hemisuccinamido methyl esters, (21) and (22), and of the 17β-acetates, (23) and (24).

III. SYNTHESIS OF THE 7-TEREPHTHALAMIDO DERIVATIVES

1°) 17β-Tetrahydropyranyloxy-3-ethylenedioxy-5-en-7β- and 7α-amines and acetamides (Fig. 5) :

The 17β-tetrahydropyranyloxy-3-ethylenedioxy-7-oximino-5-androstene (28) was prepared by condensation of hydroxylamine hydrochloride with the previously described 17β-tetrahydropyranyloxy-5-en-7 ketone (27) [9] in pyridine solution, at room temperature. All attempts to prepare the preceding ketone from the 17β-acetoxy-5-en-7-ketone (1) by saponification of the 17β-acetate group followed by acid-catalyzed addition of dihydropyran led to enolized derivatives [12].

The reduction of the conjugated 7-oxime (28) with sodium in ethanol gave a crude mixture containing the two 17β-tetrahydropyranyloxy-5-en-7β-and 7α-amines as well as their 5,6-dihydro analogs. Preparative t.l.c. on silica gel ($CHCl_3$-MeOH-NH_4OH 100:10:1) allowed the isolation of the pure 17β-tetrahydropyranyloxy-3-ethylenedioxy-5-en-7β-amine (29) (25 %) as the faster-moving product and of its 5-en-7α-epimer (30) (21 %) as the slower-moving product, which were also characterized as 7β- and 7α-acetamides, (31) and (32).

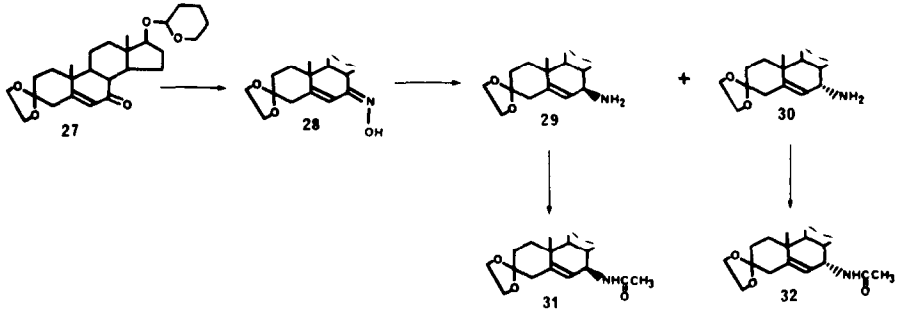


Fig. 5

2°) 17β-Tetrahydropyranyloxy-3-ethylenedioxy-5-en-7β- and 7α-terephthalamido derivatives (Fig. 6) :

In preliminary attempts, the two 17β-tetrahydropyranyloxy-5-en-7β- and 7α-

terephthalamido methyl esters, (33) and (34), were prepared in low and irreproducible yields by condensation of the 5-en-7β- and 7α-amines, (29) and (30), with the mono-mixed anhydride of terephthalic acid (1,4-benzenedicarboxylic acid) [13], followed by diazomethane esterification and preparative t.l.c. Therefore, a much more reliable procedure was devised for the purpose of introducing in one step the 7-terephthalamido methyl ester group by condensation of the 7-amine with the acid chloride of terephthalic acid monomethyl ester (see Experimental part) which gave 74-76 % yields of the above methyl esters, (33) and (34).

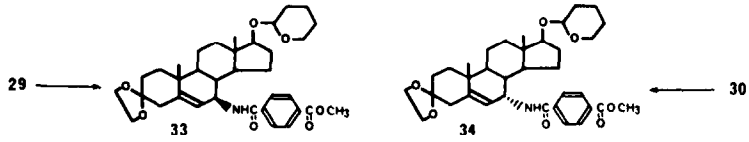
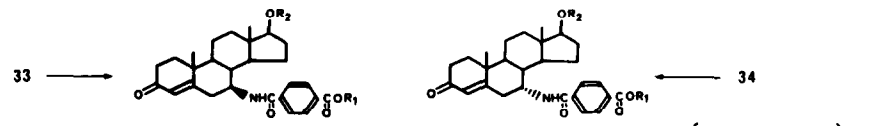


Fig. 6

As mentioned in the case of the other 3-ethylenedioxy-5-en-7α-N-acyl derivatives, the slower-moving 17β-tetrahydropyranyloxy-5-en-7α-terephthalamido methyl ester was easily separated from 5,6-dihydro contaminants as well as from the faster-moving dimethyl terephthalate [14], after t.l.c. on silica gel (petroleum ether-EtOAc 3:2, 3 developments).

3°) 4-En-3-oxo-7β- and 7α-terephthalamides and methyl esters (Fig. 7) :

The two pure 17β-hydroxy-4-en-3-oxo-7β- and 7α-terephthalamido methyl esters, (35) and (36), characterized also as 17β-acetates, (37) and (38), were obtained in nearly quantitative yields after acidolysis (HCl-dioxane) of each of the 17β-tetrahydropyranyloxy-3-ethylenedioxy-5-en-7β-and 7α-terephthalamido methyl ester precursors, (33) and (34). As described for the other 4-en-3-oxo-7-N-acyl derivatives, this acidolysis step was found to lead to an inversion of the order of the Rf values of the 7-epimers after t.l.c. on silica gel performed with petroleum ether-EtOAc or $CHCl_3$-EtOAc mixtures, thus allowing the isolation of the slower-moving 7β-epimer from mixtures containing 5,6-dihydro contaminants.



35 36 (R_1=CH_3, R_2=H)

37 38 (R_1=CH_3, R_2=$COCH_3$)

39 40 (R_1, R_2 = H)

Fig. 7

The two desired 17β-hydroxy-4-en-3-oxo-7β- and 7α-terephthalamides, (39) and (40), were obtained in nearly quantitative yields either from the corresponding 17β-acetoxy-4-en-3-oxo-7β- and 7α-terephthalamido methyl esters, (37) and (38), or from their 17β-hydroxy precursors, (35) and (36), after saponification followed by acid precipitation of the free terephthalamides. These carboxylic acids were characterized by t.l.c. on silica gel ($CHCl_3$-acetone-acetic acid 7:2:1, slower-moving 7β-epimer). Diazomethane esterification of these terephthalamides [3], followed by acetylation gave, successively, quantitative yields of the previously described 17β-hydroxy-4-en-3-oxo-7β- and 7α-terephthalamido methyl esters, (35) and (36), and 17β-acetates, (37) and (38).

IV. DETERMINATION OF THE 7β- AND 7α- CONFIGURATIONS OF THE 3-ETHYLENEDIOXY-5-EN-7-AMINES, 4-EN-3-OXO-7-AMINES AND OF THEIR 7-N-ACYL DERIVATIVES

1°) Stereochemistry of the 3-ethylenedioxy-5-en-7β- and 7α-amines and N-acyl derivatives :

The equatorial and axial conformations of the 5-en-7β- and 5-en-7α-amines and of their N-acyl derivatives were established according to the following data :

The ^{1}H-nmr spectra of all the faster-moving epimers (t.l.c. of 7-amines with $CHCl_3$-MeOH-NH_4OH mixtures and of 7-N-acyl derivatives with solvent mixtures containing EtOAc) showed a characteristic pseudo-triplet peak at 5.1-5.2 ppm corresponding to the vinylic 6-proton. The coupling constants ($J \backsim 2$ Hz) were found in agreement with the two small values which can be expected from coupling of the 6-proton with each of the two axial 7α-and 4β-protons [15], thus suggesting a 7β-orientation of these faster-moving amino derivatives. On the other hand, the vinylic 6-proton signal of all the slower-moving epimers appeared as a quartet at 5.4-5.5 ppm. The smaller coupling constant ($J_1 \backsim 2$ Hz) could still be attributed to the homoallylic coupling of the 6-proton with the 4β-proton whereas the larger coupling constant ($J_2 \backsim 5$ Hz) was in good agreement with the expected value for the coupling of the 6-proton with an equatorial 7β-proton, thus suggesting a 7α-orientation of these slower-moving amino derivatives [15]. Moreover, a broad multiplet centered at 4.2 ppm was assigned to the 7α-proton of the former 7β-N-acyl derivatives whereas a similar multiplet was found downfield at 4.4 ppm in the case of the 7α-N-acyl epimers [3] and was therefore assigned to the 7β-proton. This confirmed the preceding configurations, within the limits that the above compounds are not among the exceptions to the rule of the higher-field resonance of axial ring protons [16].

These stereochemical assignments were further corroborated by double-resonance decoupling experiments performed on the two 5-en-7β- and 7α-hemisuccinamido methyl esters, (17) and (18). Double irradiation at the NHCO proton frequency (∿ 5.7 ppm, doublet : J_{NH-7H} ∿ 10 Hz) induced a noticeable sharpening of the 7-proton multiplets of both the 7β- and 7α-epimers. A similar irradiation at the frequency of each of the latter multiplets collapsed the NHCO doublet to a singlet in both cases. This irradiation also collapsed the vinylic 6-proton quartet ($J_{6H-7\beta H}$ ∿ 5 Hz) of the 7α-epimer to a broad singlet, whereas the modification of the 6-proton pseudo-triplet of the 7β-epimer was not significant enough to allow further interpretation. Conversely, double-irradiation at the frequency of the vinylic 6-proton quartet of the 7α-epimer induced a sharpening of the 7β-proton signal whereas a similar irradiation of the 6-proton pseudo-triplet of the 7β-epimer did not significantly modify the 7α-proton multiplet. Furthermore, these decoupling experiments which strongly support the axial configuration of the slower-moving 5-en-7α-amino epimers are in full agreement with the results of similar experiments performed on 5-en-7α-azido steroids [17].

No significant shift of the 18-CH_3 signals could be detected between the two 5-en-7β- and 7α-epimers whereas the 19-CH_3 signals of the 5-en-7β-epimers were systematically observed at a slightly lower field (+ 0.01 ppm) than those of the 7α-epimers. This latter result was found to be in agreement with those of other studies concerning 5-en-7-bromo derivatives [5] and 5-en-7-hydroxy steroids [18] but contrasted with the previously mentioned characteristic upfield shift (– 0.03 ppm) of the 19-CH_3 signals of the 7β-amino derivatives of the 5α-H-series [3]. However, the methyl signal of the equatorial acetamide group of the 5-en-7β-acetamide (7) was still observed at higher field (1.92 ppm) than that of the axial 7α-acetamide (8) (1.95 ppm), as described previously in the 5α-H-series [3].

The preceding assignments of the 7β- and 7α-configurations of the 5-en-7-amino derivatives were also corroborated by the measurement of their optical rotations in dichloromethane, dioxane or ethanol solutions. The dextrorotatory rotations of all the faster-moving 7β-epimers and the strong levorotatory rotations of the 7α-epimers were in good agreement with the already established signs of the rotations of the corresponding 5α-H-7β- and 7α-epimers [3] and of the 5-en-7α-azido-, amino- and acetamido derivatives of cholesterol [17].

2°) Stereochemistry of the 4-en-3-oxo-7β- and 7α-N-acyl derivatives

As mentioned before, the acidolysis of the different 3-ethylenedioxy-5-en-7β- and 7α-N-acyl precursors into the desired 4-en-3-oxo-7β- and 7α-N-acyl deriva-

tives was always found to lead to an inversion of the order of the Rf values of the 7β- and 7α-epimer on silica gel plates developed with petroleum ether-EtOAc or $CHCl_3$-EtOAc mixtures, whereas no inversion occurred with $CHCl_3$-MeOH mixtures. This inversion restored the same order of polarities as that already established for the 5α-H-7-analogs [3], thus suggesting similar conformations of the ring B in both 4-en- and 5α-H-series. Furthermore, no correlations could be found between the inversion of the Rf values and the modifications of optical rotations of either the 3-ethylenedioxy-5-en-7β- and 7α-acetamide precursors, (7) and (8), or of the 4-en-3-oxo derivatives, (13) and (14), in the corresponding t.l.c. solvent mixtures (see Experimental part). However, eventhough a total inversion of the configurations of both the 7β- and 7α-epimers or other structural modifications [19] including the interconversion of the ring B seemed improbable in the above acidolysis step, such an assumption had nevertheless to be unambiguously confirmed by an independent assignment of the 7-orientations of the final 4-en-3-oxo-products. Therefore, the 7-configurations of the 4-en-3-oxo-7-amines and N-acyl derivatives were established in the following manner :

The ^{1}H-nmr spectrum of the slower-moving 4-en-3-oxo-7β-acetamide (13) showed the characteristic signal of the methyl group of the acetamide at a higher field (1.93 ppm) than that of the faster-moving 7α-epimer (14), which was observed at 1.95 ppm, thus suggesting 7β-equatorial-and 7α-axial configurations respectively for these two epimeric acetamides. On the other hand, the residual 7-proton of the slower-moving 7-N-acyl derivatives appeared as a broad multiplet at 3.5-4.0 ppm which was found to be partially superimposed on the methyl singlet peak of carboxymethylester groups as well as to the 17α-proton multiplet peak of 17β-hydroxy derivatives. The 7-proton of the other epimer appeared as a sharper multiplet at 4.2-4.5 ppm which was partially superimposed on the 17α-proton signal of 17β-acetates. The upfield shift of the 7-proton multiplet signal of the slower-moving epimers as well as its larger width which can be expected as the result of the greater coupling constant with axial 6β- and 8β-protons, strongly support the axial configuration of the 7-proton and therefore the equatorial 7β-orientation of the corresponding N-acyl derivatives.

These results were confirmed by double-irradiation experiments at the NHCO proton frequency ($\sim$ 5.7 ppm, doublet J_{NH-7H} $\sim$ 10 Hz) performed on the two epimeric 7-hemisuccinamido methyl esters, (23) and (24), which significantly sharpened the corresponding 7-proton multiplet, thus allowing a better separation of these signals from the other partially superimposed peaks mentioned above.

The 4-en-3-oxo-7β- and 7α-N-acyl derivatives could also be distinguished through the ^{1}H-nmr signals of their angular methyl groups. In all cases, no significant shift of the 18-CH_3 peaks was observed between the two 7-epimers, whereas the 19-CH_3 peaks of the slower-moving 7β-epimers were slightly shifted upfield (- 0.01 ppm) as compared to those of the 7α-epimers. As previously mentioned in the case of the corresponding analogs of the 5α-H-series but in contrast to that of the 5-ene precursors, this latter upfield shift seems characteristic of the equatorial 7β-epimers.

The optical rotations of the 4-en-3-oxo-7β- and 7α-amino derivatives were also measured in dichloromethane, dioxane or ethanol solutions. In all cases, the slower-moving epimers showed dextrorotatory rotations similar to those described above for the 7β-epimers of both the corresponding 5α-H- and 5-ene derivatives, thus supporting the equatorial 7β-amino configuration established from nmr spectra. On the other hand, the faster-moving epimers showed slightly levorotatory rotations either in dioxane or ethanol solution (except for compound 38), which were similar to those measured previously for the 7α-epimers of the 5α-H-series [13], thus confirming the axial 7α-configuration assigned by nmr.

However, the measurement of the optical rotations of the 4-en-3-oxo-7α-N-acyl derivatives in dichloromethane solution, as well as in other usual chlorinated solvents such as chloroform or 1,2-dichloroethane often led to significantly lesser levorotatory values than those observed in dioxane or ethanol solution, thus rendering very questionable all attempts to distinguish these 7-amino epimers by molecular rotation increment correlations only [20].

These dextrarotatory solvent shifts were found to be instantaneous, reversible and independent of concentration or temperature (4°-30°C). They were progressively decreased with decreasing proportions of chlorinated solvents. Moreover, no significant modifications of the ultraviolet (ethanol *vs* CH_2Cl_2) and infrared (KBr *vs* CH_2Cl_2) absorption properties could be observed between the 4-en-3-oxo-7α- and 7β-N-acyl derivatives, whereas no trace of 3-hydroxy-3,5-diene could be detected in the nmr spectra of the 7α-epimers. These observations ruled out the hypothesis of keto-enol tautomerism which had previously been suggested as a possible interpretation of similar changes in optical rotation properties of 3-hydroxy-3,5-dien-7-oxo steroids in chloroform solution [21]. Another comparison can be made with the progressive and reversible dextrarotatory mutarotation in chloroform of isocolchicine derivatives [22], which have a δ-amido-α,β-ethylenic ketone group similar to that of the 4-en-3-oxo-7-N-acyl steroids. It was proposed

that this mutarotation might rather be associated with the slow interconversion of two diastereoisomers resulting from hindered rotation of the two rings A and C than with the instantaneous formation of 1:1 complexes of these compounds with chloroform. As mentioned before, it is doubtful whether such an interconversion can occur in the case of the much more rigid rings A and B of the steroid molecules studied in this work (see IV,2°). Nevertheless one can speculate whether the relatively stronger solvent shifts of the 4-en-3-oxo-7α-N-acyl derivatives, as compared with those of the 7β-epimers or of the 5-en- and 5α-dihydro analogs, might result from conformational modifications of the 7-substituent, owing to the influences of solvent-dependent interactions between the axial 7α-chain and the 4-en-3-oxo group as expected from the strong solvation of most 7-N-acyl derivatives [3].

The above 7-configurations were also found in agreement with those previously assigned to the corresponding 4-en-3-oxo-7β- and 7α-hydroxy and acetoxy derivatives [11] as well as to the 4-en-3-oxo-7α-thioether alkanoic acid derivatives [19], thus confirming the above structural assignments.

The extension of this work towards the access to similar 4-en-3-oxo-7-amino derivatives in other steroid series is now in progress in this laboratory.

EXPERIMENTAL

Thin-layer chromatographies (t.l.c.) were carried out on fluorescent silica gel plates (Merck GF 254). The petroleum ether fraction employed had a bp : 45-65°C. The NH_4OH solution employed contained about 20 % NH_3 (d ≃ 0.92). The spots corresponding to 3-ethylenedioxy-5-ene derivatives were revealed under UV-light after spraying with 18N H_2SO_4. Successive developments of silica gel plates were systematically performed after changing the solvent mixtures at each time. Compounds containing ethyleneketal or tetrahydropyranyl protecting groups were systematically recrystallized in the presence of traces of pyridine. The number of recrystallizations and the solvent mixture leading to analytical samples are mentioned in parentheses after the mp and are abbreviated as follows : (cryst x n, solvent). Melting points were taken on a Leitz hot-stage microscope and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°C. Unless specified, IR spectra were recorded on a Perkin-Elmer 257 spectrometer in CCl_4 solution, UV spectra on a Zeiss DM 5 spectrometer in 95 % ethanol and ^1H-nmr spectra on a Bruker CW-80 spectrometer in $CDCl_3$ solution. Mass spectra were obtained using a VG Micromass 7070 spectrometer. Elemental analyses were determined by Service Central de Microanalyse du CNRS, Solaise-Vernaison. Unless specified analytical samples were dried for 48 h at 70°C under 10^{-3} mm Hg. In those instances where unsatisfactory elemental analyses seemed to result from a strong solvation of the crystals [3], the temperature was progressively increased (up to 200°C) thus requiring a careful control of this treatment in order to avoid thermal decomposition.

3,3'-Ethylenedioxy-7-oximino-5-androsten-17β-yl acetate, (2) :

A mixture of 3,3'-ethylenedioxy-7-oxo-5-androsten-17β-yl-acetate (1) [9] (39 g, 100 mmole) and hydroxylamine hydrochloride (10.5 g, 150 mmole) was dissolved in 2 l of pyridine and stirred overnight at room temperature. The

residue after evaporation to dryness was taken up in $CHCl_3$ which was washed with water and evaporated to give the 7-oxime (2) (39 g; 97 %), mp 228-235°C. Rf 0.50 (petroleum ether-EtOAc, 3:2) ; mp 233-236°C (cryst. x 3, MeOH) ; $[\alpha]_D$ = 169° (c, 0.5, CHCl3) ; λ max (ε) : 241 nm (16000) ; νmax : 3600-3200 (NOH), 1735 (OCO), 1640 (C=N), 1100 cm^{-1} (ether) ;
nmr : δ ppm 0.83 (3H,s,18-CH3), 1.13 (3H,s,19-CH3), 2.02 (3H,s,COCH3), 3.9 (4H, s, OCH2CH2O), 4.6 (1H, m, 17α-H), 6.5 (1H, d: J ≃ 2 Hz, 6-H), 6.8 (1H, s, NOH).
Anal. Calcd. for $C_{23}H_{33}O_5N$: C, 68.46 ; H, 8.24 ; N, 3.47.
Found : C, 68.39 ; H, 8.23 ; N, 3.49.

7β- and 7α-Amino-3,3'-ethylenedioxy-5-androsten-17β-ols, (3) and (4) :

To a solution of 7-oxime (2) (8.1 g, 20 mmole) in 1.7 l of absolute EtOH was added 140 g of sodium during 2 h. The mixture was refluxed for 1 h, then 1.5 l of water was added. The alcohol was distilled off, the mixture was cooled and the product was collected by filtration. The crude residue was taken up in a CH_2Cl_2-water mixture. The pH was first brought to 5-6 with conc. HCl and then to 9-10 with NH_4OH. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to give a mixture of 7-amines (7.2 g). Preparative t.l.c. on silica gel of this crude mixture ($CHCl_3$-MeOH-NH_4OH 100:10:1, x 3 dev.) allowed the separation of three fractions : a) the faster-moving spot (1.8 g, 26 %) was identified as the pure 5-en-7β-amine (3) ; b) the intermediate spot (2.1 g, 30 %) was found to contain a mixture of 5α-dihydro-7β-amine (5) (major product) and 7α-amine (6) which were identified after acetylation (vide infra) ; c) the slower-moving spot (1.2 g, 17 %), identified as the 5-en-7α-amine (4), had an Rf value very close to that of the above intermediate spot, thus requiring several successive chromatographies in order to obtain the pure product. These 7-amines were found to take up carbon dioxide from the air [8] whereas a noticeable amount of starting 5-en-7-ketone (1) was often formed after prolonged storage in a vacuum dessicator. All attempts to purify the 17β-hydroxy-5-en-7-amines by sublimation resulted in a partial decomposition. On the other hand, no satisfactory elemental analyses of the corresponding hydrochlorides or acetic acid salts could be obtained owing probably to the difficulties encountered when drying these salts without decomposition.

7β-epimer (3)
Rf 0.3 ($CHCl_3$-MeOH-NH_4OH 100:10:1) ; mp 137-145°C (dec.) (crude powder) ; $[\alpha]_D$ = + 16° (c, 0.3, CH_2Cl_2 + traces of NH4OH), + 22° (c, 0.2, 95 % EtOH + traces of NH4OH) ; νmax (CH_2Cl_2) : 3650-3600 (OH, NH2), 1100 cm^{-1} (ether) ;
nmr : δppm 0.76 (3H, s, 18-CH3), 1.04 (3H, s, 19-CH3), 3.7 (1H, m, 17α-H), 3.9 (1H, s, OCH2CH2O), 5.2 (1H, t : J ≃ 2 Hz, 6-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 347 (M^+, 14), 330 (24), 302 (48), 286 (51), 145 (7), 99 (75), 83 (100).

7α-epimer (4)
Rf 0.2 (CHCl3-MeOH-NH4OH 100:10:1) ; mp 160-166°C (dec.) (white powder) ; $[\alpha]_D$ = - 53° (c, 0.2, CH_2Cl_2 + traces of NH4OH), - 53° (c, 0.2, 95 % EtOH + traces of NH4OH) ; νmax (CH_2Cl_2) : 3650-3600 (OH, NH2), 1100 cm^{-1} (ether) ;
nmr : δ ppm 0.76 (3H, s, 18-CH3), 1.03 (3H, s, 19-CH3), 3.7 (1H, m, 17α-H), 3.9 (1H, s, OCH2CH2O), 5.6 (1H, q: J6H,7βH ≃ 5 Hz, J6H,4αH ≃ 2 Hz, 6-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 347 (M^+, 9), 330 (24), 302 (18), 286 (18), 99 (100), 83 (14).

7β- and 7α-Acetamido-3,3'-ethylenedioxy-5-androsten-17β-yl acetates, (7) and (8) :

A solution of pure 5-en-7β-amine (3) or 5-en-7α-amine (4) (0.35 g, 1.0 mmole) in 10 ml of pyridine-acetic anhydride 3:1 (v/v) mixture was allowed to stand at room temperature overnight and was then evaporated to dryness under reduced pressure. The crude residue was purified by preparative t.l.c. on silica gel (EtOAc,

x 5 dev.) to give the 17β-acetoxy-5-en-7β-acetamide (7) (0,33 g, 77 %) or 7α-acetamide (8) (0.34 g, 79 %). The 7α-acetamide (8) was also isolated after acetylation of the crude mixture of 7-amines by t.l.c. on silica gel (petroleum ether-EtOAc 1:3, x 8 dev.) as the slower-moving product whereas the intermediate spot was found to contain the 5α-dihydro-7β-acetamide (9) as the major product (vide infra). The faster-moving spot was found to be an inseparable mixture of 5α-dihydro-7α-acetamide (10) (vide infra) and 5-en-7β-acetamide (8).

7β-epimer (7)

Rf 0.60 (EtOAc or CHCl3-EtOAc 1:3, x 5 dev.), 0.55 (petroleum ether-EtOAc 1:3, x 8 dev.), 0.48 (CHCl3-MeOH 10:1) ; mp 295-297°C (cryst. x 4, CH2Cl2-MeOH) ; $[\alpha]_D$ = + 68° (c, 0.2, CH2Cl2), + 63° (c, 0.2, dioxane), + 67° (c, 0.3, CHCl3-MeOH 10:1), + 73° (c, 0.2, EtOAc) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δppm 0.80 (3H, s, 18-CH3), 1.05 (3H, s, 19-CH3), 1.92 (3H, s, NHCOCH3), 2.02 (3H, s, OCOCH3), 3.9 (4H, s, OCH2CH2O), 4.2 (1H, m, 7α-H), 4.6 (1H, m, 17α-H), 5.1 (1H, t: J6H,7αH and J6H,4βH ≃ 2 Hz, 6-H), 5.2 (1H, d : J ≃ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 431 (M^+, 36), 386 (10), 372 (M^+-7-substituent, 7), 370 (28), 344 (10), 328 (4), 99 (100).

No satisfactory elemental analysis could be obtained for this compound [3].

7α-epimer (8)

Rf 0.40 (EtOAc or CHCl3-EtOAc 1:3, x 5 dev.), 0.30 (petroleum ether-EtOAc 1:3, x 8 dev.), 0.54 (CHCl3-MeOH 10:1) ; mp 120-130°C — resin (cryst. x 4, ether-pentane) ; $[\alpha]_D$ = - 153° (c, 0.3, CH2Cl2), - 160° (c, 0.3, dioxane), - 170° (c, 0.3, CHCl3-MeOH 10:1), - 158° (c, 0.3, CHCl3-EtOAc 1:3) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δ ppm 0.80 (3H, s, 18-CH3), 1.04 (3H, s, 19-CH3), 1.95 (3H, s, NHCOCH3), 2.02 (3H, s, OCOCH3), 3.9 (4H, s, OCH2CH2O), 4.4 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 5.3 (1H, q: J6H,7βH ≃ 5 Hz, J6H,4βH ≃ 2 Hz, 6-H), 5.3 (1H, d: J ≃ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 431 (M^+, 12), 386 (3), 372 (M^+-7-substituent, 3), 370 (10), 344 (5), 328 (3), 99 (100).

Anal. Calcd. for $C_{25}H_{37}O_5N$: C, 69.57 ; H, 8.64 ; N, 3.25.
Found : C, 69.82 ; H, 8.74 ; N, 3.16.

7β- and 7α-Acetamido-3,3'-ethylenedioxy-5α-androstan-17β-yl acetates, (9) and (10) :

The 5α-dihydro-7β- and 7α-acetamides, (9) and (10), mentioned above as contaminants of the 5-ene analogs have been identified by comparison with reference samples prepared by acetylation and t.l.c. on silica gel (petroleum ether-EtOAc 1:3, x 8 dev., faster-moving 7α-epimer) of the crude 7β- and 7α-amino-3-ethylenedioxy-5α-androstan-17β-ol mixture, (5) and (6), obtained as described previously [3] except for the final acidolysis step which was omitted.

7β-epimer (9)

Rf 0.50 (EtOAc or CHCl3-EtOAc 1:3, x 5 dev.), 0.45 (petroleum ether-EtOAc 1:3, x 8 dev.), 0.48 (CHCl3-MeOH 10:1) ; mp 260-262°C (cryst. x 4, CH2Cl2-ether) ; $[\alpha]_D$ = + 34° (c, 0.1, CH2Cl2), + 24° (c, 0.1, dioxane) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δppm 0.77 (3H, s, 18-CH3), 0.80 (3H, s, 19-CH3), 1.92 (3H, s, NHCOCH3), 2.02 (3H, s, OCOCH3), 3.7 (1H, m broad, 7α-H), 3.9 (4H, s, OCH2CH2O), 4.5 (1H, m, 17α-H), 5.5 (1H, d: J ≃ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 433 (M^+, 2), 347 (16), 182 (75), 99 (100).

Anal. Calcd. for $C_{25}H_{39}O_5N$: C, 69.25 ; H, 9.07 ; N, 3.23.
Found : C, 69.51 ; H, 9.31 ; N, 3.43.

7α-epimer (10)

Rf 0.60 (EtOAc or CHCl3-EtOAc 1:3, x 5 dev.), 0.55 (petroleum ether-EtOAc 1:3,

x 8 dev.), Rf identical to those of the 5-en-7β-acetamide (9), 0.54 ($CHCl_3$-MeOH 10:1) ; mp 125-130°C → resin (cryst. x 4, ether-pentane) ; $[\alpha]_D$ = -21° (c, 0.4, CH_2Cl_2), - 29° (c, 0.4, dioxane) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;
nmr : δ ppm 0.78 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3), 2.01 (3H, s, $NHCOCH_3$), 2.02 (3H, s, $OCOCH_3$), 3.9 (4H, s, OCH_2CH_2O), 4.2-4.7 (1H, m, 7β-H), 4.5 (1H, m, 17α-H), 5.7 (1H, d: J ≃ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 433 (M^+, 7), 374 (20), 182 (80), 99 (100).
Anal. Calcd. for $C_{25}H_{39}O_5N$: C, 69.25 ; H, 9.07 ; N, 3.23.
Found : C, 69.22 ; H, 8.89 ; N, 3.06.

7β- and 7α-Amino-17β-hydroxy-4-androsten-3-ones, (11) and (12) :

The pure 3-ethylenedioxy-5-en-7β-amine (3) or 7α-amine (4) (0.35 g, 1.0 mmole) were dissolved in 20 ml of a dioxane-water 9:1 (v/v) mixture which was acidified to pH 2 with conc. HCl. The reaction mixture was stirred at 20°C until the reaction was complete (≃ 1 h). Then, the pH was brought to 6 with $NaHCO_3$ and the solvent was evaporated at 20°C under reduced pressure. The residue was stirred at 4°C with a mixture of 20 ml CH_2Cl_2 and 20 ml of water, brought to pH 2-3 with HCl. The organic layer was discarded and the cold aqueous layer was brought to pH 9 with NH_4OH. Extraction of the aqueous layer with CH_2Cl_2 followed by evaporation at 20°C under reduced pressure gave the 4-en-3-oxo-7β-amine (11) (0.23 g, 74 %) or 7α-amine (12) (0.25 g, 80 %).

The acidolysis of 3-ethylenedioxy-5-en-7-amines contaminated with 5,6-dihydro by-products led to a mixture of the above 4-en-3-oxo-7-amines and of the previously described 3-oxo-5α-dihydro-7β- and 7α-amines [3]. Preparative t.l.c. on silica gel ($CHCl_3$-MeOH-NH_4OH 100:10:1) allowed the isolation of three UV-adsorbing spots : a) the faster-moving product (5-10 %) was identified as 17β-hydroxy-4,6-androstadien-3-one [9]. The amount of 3-dienone was lowered under 5 % when the silica gel plates were first neutralized by the above NH_4OH-containing solvent. b) the intermediate product corresponding to the above 4-en-3-oxo-7β- or 7α-amine could not be eluted from the silica gel without contamination with the 3-dienone (5-10 %). Therefore, these 7-amines were solubilized in water as the corresponding hydrochlorides after addition of HCl (pH 2-3) whereas the 3-dienone was extracted with CH_2Cl_2. The pure free 7-amines were then regenerated after addition of NH_4OH, followed by CH_2Cl_2 extraction and evaporation of the solvent at 20°C under reduced pressure. c) the slower-moving product (≃ 30 %) was found to contain the previously described 3-oxo-5α-dihydro-7β- and 7α-amines which were also identified as the corresponding 7-acetamides [3]. All attempts to purify the free 4-en-3-oxo-7-amines by sublimation (150-200°C, 10^{-3} mm Hg) resulted in yellowish decomposed products.

7β-epimer (11)
Rf 0.28 ($CHCl_3$-MeOH-NH_4OH 100:10:1) ; mp 95-100°C (amorphous powder) ; $[\alpha]_D$ = + 91° (c, 0.2, CH_2Cl_2 + traces of NH4OH), + 87° (c, 0.2, 95 % EtOH + traces of NH4OH) ; λ max (ε) 240 nm (12100) ; νmax (CH_2Cl_2) : 3650-3600 (OH, NH_2), 1670 (conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.80 (3H, s, 18-CH_3), 1.20 (3H, s, 19-CH_3), 3.4-3.7 (2H, m broad, 17α-H and 7α-H), 5.8 (1H, s, 4-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 303 (M^+, 95), 288 (34), 286 (51), 271 (15), 268 (9), 253 (14), 242 (9), 232 (10), 227 (7), 180 (60), 151 (100), 136 (40), 133 (22), 122 (31), 107 (25), 93 (22), 79 (29).

7α-epimer (12)
Rf 0.28 ($CHCl_3$-MeOH-NH_4OH 100:10:1) ; mp 125-128°C (cryst. x 8, CH_2Cl_2-ether + traces of NH4OH) ; $[\alpha]_D$ = + 63° (c, 0.1, CH_2Cl_2 + traces of NH4OH), + 52° (c, 0.1, 95 % EtOH + traces of NH4OH) ; λ max (ε)242 nm (12300) ; ν max

(CH2Cl2) : 3650-3600 (OH, NH2), 1670 (conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm 0.80 (3H, s, 18-CH3), 1.20 (3H, s, 19-CH3), 3.2 (1H, m, 7β-H), 3.7 (1H, m, 17α-H), 5.9 (1H, s, 4-H) ;
mass spectrum (70eV), m/e (rel. intensity) 303 (M^+,80), 288 (38), 286 (95), 271 (19), 268 (17), 253 (21), 242 (13), 232 (10), 227 (13), 180 (55), 151 (100), 107 (37), 93 (27).

7β- and 7α-Acetamido-3-oxo-4-androsten-17β-yl acetates, (13) and (14) :

The pure 3-ethylenedioxy-5-en-7β -acetamide (7) or the corresponding 7α-epimer (8) (0.22 g, 0.51 mmole) were dissolved in 20 ml of a dioxane-water 9:1 mixture, which was acidified to pH 2 with conc. HCl. The reaction mixture was stirred at 30°C until the starting-product had totally disappeared on t.l.c.. Then, the pH was brought to 6 with NaHCO3 and the solvent was evaporated at 30°C under reduced pressure. The pure 4-en-3-oxo 7β-acetamide (13) (0.16 g, 81 %), or the corresponding 7α-epimer (14) (0.17 g, 86 %) were isolated by t.l.c. on silica gel (EtOAc, x 4 dev.). This acidolysis was also performed on the acetylation product of the crude mixture of 3-ethylenedioxy-7-amines. The pure 4-en-3-oxo-7β-acetamide (13) was isolated as the slower-moving product after preparative t.l.c., as described above. The two next spots were identified as the 3-oxo-5α-dihydro-7β-acetamide [3] and as an inseparable mixture of 3-oxo-5α-dihydro-7α-acetamide [3] and 4-en-3-oxo-7α-acetamide (14), respectively. The two 4-en-3-oxo-7β- and 7α-acetamides were also obtained in nearly quantitative yields after acetylation of the 4-en-3-oxo-7β- and 7α-amines, (11) and (12), with an acetic anhydride-pyridine 1:5 mixture, overnight at room temperature, followed by evaporation of the excess of reagents under a stream of nitrogen at 20°C.

7β-epimer (13)

Rf 0.37 (EtOAc, x 4 dev.) vs 0.43 and 0.57 for the 7β- and 7α-acetamido-3-oxo-5α-androstan-17β-yl acetates [3], 0.30 (CHCl3-EtOAc 1:3, x 4 dev.) vs 0.38 and 0.48 for the preceding 5α-dihydro analogs, 0.32 (CHCl3-MeOH 10:1) ; mp 168-171°C, then 225-235°C (cryst. x 4, CH2Cl2-ether), 245-248°C (after sublimation at 200°C under 10^{-3} mm Hg) ; $[\alpha]_D$ = + 73° (c, 0.1, CH2Cl2), + 60° (c, 0.1, dioxane), + 64° (c, 0.3, CHCl3-MeOH 10:1), + 52° (c, 0.4, CHCl3-EtOAc 1:3) ; λ max (ε) :242-243 nm (14600) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm 0.85 (3H, s, 18-CH3), 1.21 (3H, s, 19-CH3), 1.93 (3H, s, NHCOCH3), 2.02 (3H, s, OCOCH3), 3.5-4.0 (1H, m, 7α-H), 4.6 (1H, m, 17α-H), 5.6 (1H, d: J ≃ 10 Hz, NHCO), 5.7 (1H, s, 4-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 387 (M^+, 46), 345 (8), 344 (6), 328 (M^+-7-subst., 89), 313 (19), 286 (62), 284 (15), 268 (74), 253 (30), 136 (76), 133 (100).

Anal. Calcd. for $C_{23}H_{33}O_4N$ = C, 71.29 ; H, 8.58 ; N, 3.61.
Found : C, 71.30 ; H, 8.74 ; N, 3.51.

7α-epimer (14)

Rf 0.57 (EtOAc, x 4 dev.), 0.48 (CHCl3-EtOH 1:3, x 4 dev.), 0.47 (CHCl3-MeOH 10:1), Rf identical to those of the 3-oxo-5α-dihydro-7α-acetamide [3] ; mp 218-227°C (cryst. x 4, CH2Cl2-ether) ; $[\alpha]_D$ = - 4° (c, 0.9, CH2Cl2), - 18° (c, 0.4, dioxane), - 20° (c, 0.3, CHCl3-MeOH 10:1), - 17° (c, 0.3, CHCl3-EtOAc 1:3) ; λmax (ε) : 240-241 nm (13000) ; ν max : 3450-3300 (NH), 1735 (OCO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.85 (3H, s, 18-CH3), 1.23 (3H, s, 19-CH3), 1.95 (3H, s, NHCOCH3), 2.02 (3H, s, OCOCH3), 2.6 (4H, m, $COCH_2CH_2CO$), 4.2-4.7 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 5.3 (1H, d: J ≃ 10 Hz, NHCO), 5.7 (1H, s, 4-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 387 (M^+, 100), 345 (7), 344 (6), 328 (M^+-7-subst., 91), 313 (43), 286 (52), 284 (14), 268 (63), 253 (30), 136 (63), 133 (83).

Anal. Calcd. for $C_{23}H_{33}O_4N$: C, 71.29 ; H, 8.58 ; N, 3.61.
Found : C, 71.08 ; H, 8.25 ; N, 3.40.

7β- and 7α-Hemisuccinamido-3,3'-ethylenedioxy-5-androsten-17β-ol methyl esters, (15) and (16) :

A solution of pure 5-en-7β-amine (3) or 5-en-7α -amine (4) (1.39 g, 4.0 mmole) and succinic anhydride (1.2 g, 12 mmole) in 50 ml of pyridine, was refluxed for 3 h. The organic solvent was distilled off and the crude residue was heated in an oil bath at 150°C under reduced pressure (10^{-3} mm Hg) in order to eliminate the excess of succinic anhydride by sublimation on a cooled finger. The residue was then refluxed overnight in 50 ml of a 5 % KOH solution in aqueous MeOH in order to saponify the remaining succinic anhydride and the 17β-hemisuccinate contaminants. The reaction mixture was brought to pH 7 with HCl and the solvent was evaporated under reduced pressure. The aqueous residue was cooled at 4°C and was acidified at pH 3-4. The precipitate was extracted with a $CHCl_3$-EtOH 10:1 mixture which was washed with water and evaporated to give the crude acid (1.2 g, Rf = 0.3, silica gel plates, $CHCl_3$-acetone-acetic acid 7:2:1). The crude acid was suspended in 50 ml of $CHCl_3$ and was treated with an excess of ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen under a well-ventilated hood, and the solvents were evaporated. The residue was purified by t.l.c. on silica gel to give the hemisuccinamido methyl esters, (15) (0.9 g, 49 %) or (16) (1.1 g, 59 %).

7β-epimer (15)

Rf 0.50 ($CHCl_3$-EtOAc 1:3, x 4 dev.), 0.36 ($CHCl_3$-MeOH 10:1) ; mp 256-257°C (cryst. x 4, CH_2Cl_2-ether) ; $[\alpha]_D$ = + 68° (c, 0.1, CH_2Cl_2), + 61° (c, 0.1, dioxane) ; νmax (CH_2Cl_2) : 3640-3300 (OH, NH), 1735 (COO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δppm 0.75 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 2.6 (4H, m, $COCH_2CH_2CO$), 3.6 (1H, m, 17α-H), 3.7 (1H, s, $COOCH_3$), 3.9 (4H, s, OCH_2CH_2O), 4.2 (1H, m, 7α-H), 5.1 (1H, t: J6H,7αH and J6H,4βH ≃ 2 Hz, 6-H), 5.3 (1H, d: J ≃ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 461 (M^+, 31), 416 (10), 400 (35), 346 (10), 330 (M^+-7-substituent, 10), 302 (26), 286 (17), 269 (2), 254 (1), 229 (2), 132 (3), 115 (14), 99 (100).

No satisfactory elemental analysis could be obtained for this compound [3].

7α-epimer (16)

Rf 0.40 ($CHCl_3$-EtOAc 1:3, x 4 dev.), 0.40 ($CHCl_3$-MeOH 10:1) ; mp 183-187°C (cryst. x 4, CH_2Cl_2-ether) ; $[\alpha]_D$ = - 155° (c, 0.9, CH_2Cl_2), - 160° (c, 0.9, dioxane) ; νmax (CH_2Cl_2) : 3640-3300 (OH, NH), 1735 (COO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δ ppm 0.75 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 2.6 (4H, m, $COCH_2CH_2CO$), 3.6 (1H, m, 17α-H), 3.7 (3H, s, $COOCH_3$), 3.9 (4H, s, OCH_2CH_2O), 4.4 (1H, m, 7β-H), 5.3 (1H, q: J6H,7βH ≃5 Hz, J6H,4βH ≃ 2 Hz, 6-H), 5.6 (1H, d: J ≃ 10 Hz, NHCO) ;

mass spectrum (70eV), m/e (rel. intensity) 461 (M^+,13), 416(3), 400(10), 346(3), 330 (M^+-7-substituent, 5), 302(6), 286(5), 269(1), 254(7), 229(1), 132(7), 115(8), 99(100).

Anal. Calcd. for $C_{26}H_{39}O_6N$: C, 67.65 ; H, 8.52 ; N, 3.03.
Found : C, 67.92 ; H, 8.69 ; N, 3.03.

7β- and 7α-Hemisuccinamido-3,3'-ethylenedioxy-5-androsten-17β-yl-acetate methyl esters, (17) and (18) :

A solution of pure 17β-hydroxy-7β-hemisuccinamido methyl ester (15) or of its 7α-epimer (16) (0.23 g, 0.50 mmole) in 10 ml of pyridine-acetic anhydride 5:1 mixture was allowed to stand at room temperature overnight and was then evaporated to dryness under reduced pressure. The pure 17β-acetates, (17) (0.21 g, 83 %) and (18) (0.22 g, 87 %), were obtained by t.l.c. on silica gel (EtOAc). The 5-

en-7α-hemisuccinamido methyl ester (18) was also obtained either from the impure 5-en-7α-amine fraction (4) contaminated with 5α-dihydro-7β- and 7α-amines, (5) and (6), or from the crude mixture of 7-amines. The pure 5-en-7α-hemisuccinamido methyl ester was isolated as the slower-moving product after t.l.c. on silica gel (petroleum ether-EtOAc 1:3, x 4 dev.). The two next spots were identified (vide infra) as the 5α-dihydro-7β-hemisuccinamido methyl ester (19) and as an inseparable mixture of 5α-dihydro-7α-hemisuccinamido methyl ester (20) and 5-en-7β-hemisuccinamido methyl ester (17), respectively.

7β-epimer (17)

Rf 0.40 (EtOAc or CHCl3-EtOAc 1:3), 0.60 (petroleum ether-EtOAc 1:3, x 4 dev.), 0.63 (CHCl3-MeOH 10:1); mp 189-191°C (cryst. x 5, CH2Cl2-ether); $[\alpha]_D$ = + 64° (c, 0.6, CH2Cl2), + 61° (c, 0.6, dioxane), + 60° (c, 0.4, 95 % EtOH); νmax : 3440-3300 (NH), 1735 (COO, OCO), 1680 (NHCO), 1100 cm^{-1} (ether);

nmr : δ ppm 0.80 (3H, s, 18-CH3), 1.05 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 2.6 (4H, m, COCH2CH2CO), 3.7 (3H, s, COOCH3), 3.9 (4H, s, OCH2CH2O), 4.4 (1H, m, 17α-H), 5.1 (1H, t: J6H,7αH and J6H,4βH ∿ 2 Hz, 6-H), 5.3 (1H, d : J ∿ 10 Hz, NHCO);

mass spectrum (70 eV), m/e (rel. intensity) 503 (M^+, 16), 458 (5), 442 (19), 388 (4), 372 (M^+-7-substituent, 15), 344 (11), 328 (13), 133 (10), 115 (14), 99 (100).

Anal. Calcd. for $C_{28}H_{41}O_7N$: C, 66.77 ; H, 8.21 ; N, 2.78.
Found : C, 66.68 ; H, 8.25 ; N, 2.63.

7α-epimer (18)

Rf 0.35 (EtOAc or CHCl3-EtOAc 1:3), 0.40 (petroleum ether-EtOAc 1:3, x 4 dev.), 0.63 (CHCl3-MeOH 10:1), superimposed to the 7β-epimer ; mp 85-90°C → resin (cryst. x 4, ether-hexane) ; $[\alpha]_D$: - 154° (c, 0.1, CH2Cl2), - 160° (c, 0.1, dioxane), - 156° (c, 0.1, 95 % EtOH) ; νmax : 3450-3300 (NH), 1735 (OCO, COO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δ ppm 0.80 (3H, s, 18-CH3), 1.04 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 2.6 (4H, m, COCH2CH2CO), 3.7 (3H, s, COOCH3), 3.9 (4H, s, OCH2CH2O), 4.2 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 5.3 (1H, t: J6H,7βH and J6H,4βH ∿ 2 Hz, 6-H), 5.5 (1H, d: J ∿ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 503 (M^+, 7), 458 (2), 442 (6), 388 (1), 372 (M^+-7-substituent, 6), 344 (3), 328 (4), 133 (7), 115 (9), 99 (100).

Anal. Calcd. for $C_{28}H_{41}O_7N$: C, 66.77 ; H, 8.21 ; N, 2.78.
Found : C, 66.52 ; H, 8.21 ; N, 2.68.

7β- and 7α-Hemisuccinamido-3,3'-ethylenedioxy-5α-androstan-17β-yl acetate methyl esters, (19) and (20) :

The 5α-dihydro-7β- and 7α-hemisuccinamido methyl esters, (19) and (20), mentioned above as contaminants of the 5-ene analogs have been identified by comparison with reference samples prepared by hemisuccinoylation, esterification and t.l.c on silica gel (petroleum ether-EtOAc 1:3, x 4 dev., faster-moving 7α-epimer) of the crude 7β- and 7α-amino-3-ethylenedioxy-5α-androstan-17β-ol mixture, (5) and (6), as described above for the synthesis of the compounds (17) and (18).

7β-epimer (19)

Rf 0.39 (EtOAc or CHCl3-EtOAc 1:3), 0.50 (petroleum ether-EtOAc 1:3, x 4 dev.) ; mp 184-186°C (cryst. x 4, CH2Cl2-ether) ; $[\alpha]_D$ = + 25° (c, 0.2, CH2Cl2), + 20° (c, 0.2, dioxane) ; ν max : 3450-3300 (NH), 1735 (COO, OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;

nmr : δ ppm 0.77 (3H, s, 18-CH3), 0.80 (3H, s, 19-CH3), 2.6 (4H, m, COCH2CH2CO), 2.03 (3H, s, OCOCH3), 3.7 (3H, s, COOCH3 and 1H, m, 7α-H), 4.5 (1H, m, 17α-H), 5.7 (1H, d: J ∿ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 505 (M^+, 1), 474 (2), 460 (1), 406 (1), 374 (M^+-7-substituent, 18), 254 (49), 132 (28), 115 (11), 99 (100).

No satisfactory elemental analysis could be obtained for this compound [3].

7α-epimer (20)
Rf 0.40 (EtOAc or CHCl3-EtOAc 1:3), 0.60 (petroleum ether-EtOAc 1:3, x 4 dev.), Rf identical to those of the 5-en-7β-hemisuccinamido methyl ester (17) ; mp 85-90°C → resin (cryst. x 4, isopropylether) ; $[\alpha]_D$ = - 14° (c, 0.1, CH_2Cl_2), - 16° (c, 0.1, dioxane) ; ν max : 3450-3300 (NH), 1735 (COO, OCO), 1680 (NHCO), 1100 cm^{-1} (ether) ;
nmr : δ ppm 0.76 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3), 2.03 (3H, s, $OCOCH_3$), 2.6 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$), 3.9 (4H, s, OCH_2CH_2O), 4.2-4.7 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 6.0 (1H, d: J ≃ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 505 (M^+, 8), 474 (2), 460 (8), 406 (1), 374 (M^+-7-substituent, 18), 254 (49), 132 (24), 115 (27), 99 (100).
No satisfactory elemental analysis could be obtained for this compound [3].

7β- and 7α-Hemisuccinamido-17β-hydroxy-4-androsten-3-one methyl esters, (21) and (22) :

The pure 3-ethylenedioxy-5-en-7β-hemisuccinamido methyl ester (15) or the corresponding 7α-epimer (16) (0.23 g, 0.50 mmole) were dissolved in 20 ml of a dioxane-water 9:1 mixture and acidified to pH 2 with HCl. The reaction mixture was stirred at 30°C until the starting-product had completely disappeared on t.l.c.. Then, the pH was brought to 6 with $NaHCO_3$ and the solvent was evaporated at 30°C under reduced pressure. The pure 4-en-3-oxo-7β or 7α-hemisuccinamido methyl esters, (21) (0.16 g, 76 %) and (22) (0.18 g, 86 %), were isolated by t.l.c. on silica gel ($CHCl_3$-MeOH 10:1). No preparative t.l.c. could be performed with solvent mixtures containing EtOAc, owing to unavoidable crystallization of the products on the plates. However, this separation could be better performed on the corresponding 17β-acetates, (23) and (24) (vide infra). These two 7-hemisuccinamido methyl esters were also obtained after diazomethane esterification of the two corresponding carboxylic acids, (25) and (26), suspended in $CHCl_3$. The use of MeOH must be avoided in this esterification in order to prevent ring-enlargement reactions [3].

7β-epimer (21)
Rf 0.42 (EtOAc, x 4 dev.), 0.40 ($CHCl_3$-MeOH 10:1) ; mp 193-195°C (cryst. x 4, CH_2Cl_2-ether) ; $[\alpha]_D$ = + 52° (c, 0.2, CH_2Cl_2), + 45° (c, 0.2, dioxane), + 45° (c, 0.2, 95 % EtOH) ; λ max (ε) : 242-244 nm (14200) ; ν max (CH_2Cl_2) : 3640-3300 (OH, NH), 1735 (COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.80 (3H, s, 18-CH_3), 1.21 (3H, s, 19-CH_3), 3.7 (1H, m, 17α-H and 1H, m, 7α-H), 3.7 (3H, s, $COOCH_3$), 5.6 (1H, d: J ≃ 10 Hz, NHCO), 5.7 (1H, s, 4-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 417 (M^+, 37), 386 (10), 303 (8), 302 (14), 286 (M^+-7-substituent, 100), 271 (21), 268 (14), 253 (10), 242 (11), 136 (38), 133 (15), 132 (20), 115 (34).
Anal. Calcd. for $C_{24}H_{35}O_5N$: C, 69.03 ; H, 8.45 ; N, 3.35.
Found : C, 69.08 ; H, 8.47 ; N, 3.26.

7α-epimer (22)
Rf 0.48 (EtOAc, x 4 dev.), 0.41 ($CHCl_3$-MeOH 10:1) ; mp 149-153°C (cryst. x 4, acetone-ether) ; $[\alpha]_D$ = - 10° (c, 0.4, CH_2Cl_2), - 25° (c, 0.4, dioxane), - 36° (c, 0.2, 95 % EtOH) ; λ max (ε) : 241-243 nm (14000) ; ν max (CH_2Cl_2) : 3640-3300 (OH, NH), 1735 (COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.80 (3H, s, 18-CH_3), 1.22 (3H, s, 19-CH_3), 3.7 (1H, m, 17α-H and 3H, s, $COOCH_3$), 4.3 (1H, m, 7β-H), 5.7 (1H, s, 4-H), 6.1 (1H, d: J ≃ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 417 (M^+, 78), 386 (4), 303 (10), 302 (21), 286 (M^+-7-substituent, 100), 271 (38), 268 (12), 253 (10), 242 (11) 136 (32), 133 (16), 132 (26), 115 (50).
Anal. Calcd. for $C_{24}H_{35}O_5N$: C, 69.03 ; H, 8.45 ; N, 3.35.
Found : C, 68.80 ; H, 8.73 ; N, 3.04.

7β- and 7α-Hemisuccinamido-3-oxo-4-androsten-17β-yl acetate methyl esters, (23) and (24) :

A solution of pure 3-ethylenedioxy-5-en-7β-hemisuccinamido methyl ester (17) or of the corresponding 7α-epimer (18) (0.50 g, 1.0 mmole) in 50 ml of a dioxane-water 9:1 mixture was acidified to pH 2 with conc. HCl, as described above for the 17β-alcohols (21) and (22). The pure 4-en-3-oxo-7β- or 7α-hemisuccinamido methyl esters, (23) (0.38 g, 83 %) and (24) (0.39 g, 85 %), were isolated by t.l.c. (EtOAc, x 2 dev.). These two hemisuccinamido methyl esters were also obtained after acetylation (pyridine-acetic anhydride 5:1) of the pure 17β-alcohols, (21) and (22). As mentioned above for the 7-acetamides, (13) and (14), this acidolysis could also be performed on the mixture of 17β-acetoxy-7-hemisuccinamido methyl esters obtained from the crude mixture of 7-amines. The pure 4-en-3-oxo-7β-hemisuccinamido methyl ester (23) was isolated as the slower-moving product after t.l.c. on silica gel (petroleum ether-EtOAc 1:1, x 10 dev.). The two next spots, contained the 3-oxo-5α-dihydro-7β-hemisuccinamido methyl ester analog [3] and an inseparable mixture of 3-oxo-5α-dihydro-7α-hemisuccinamido methyl ester [3] and 4-en-3-oxo-7α-hemisuccinamido methyl ester (24).

7β-epimer (23)

Rf 0.35 (EtOAc or petroleum ether-EtOAc 1:5, x 2 dev.) vs 0.41 and 0.48 for the 7β- and 7α-hemisuccinamido-3-oxo-5α-androstan-17β-yl-acetate methyl esters [3], 0.32 (petroleum ether-EtOAc 1:1, x 10 dev.) vs 0.48 and 0.59 for the preceding 5α-dihydro analogs, 0.70 (CHCl3-MeOH 10:1) ; mp 172-174°C (cryst. x 4, MeOH-ether-pentane) ; $[\alpha]_D$ = + 59° (c, 0.2, CH_2Cl_2), + 51° (c, 0.2, dioxane), + 47° (c, 0.2, 95 % EtOH), + 58° (c, 0.2, CHCl3-MeOH 10:1), + 39° (c, 0.3, EtOAc) ; λ max (ε) : 242-244 nm (14800) ; ν max : 3450-3300 (NH), 1735 (OCO, COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;

nmr : δppm 0.85 (3H, s, 18-CH3), 1.21 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 2.6 (4H, m, $COCH_2CH_2CO$), 3.7 (1H, m, 7α-H and 3H, s, COOCH3), 4.6 (1H, m, 17α-H), 5.6 (1H, d: J ∿ 10 Hz, NHCO), 5.7 (1H, s, 4-H) ;

mass spectrum (70 eV), m/e (rel. intensity) 459 (M^+, 38), 428 (13), 399 (3), 345 (17), 344 (30), 328 (M^+-7-substituent, 77), 313 (27), 286 (61), 284 (16), 268 (80), 253 (40), 136 (94), 133 (100), 115 (162).

Anal. Calcd. for $C_{26}H_{37}O_6N$: C, 67.95 ; H, 8.12 ; N, 3.05.
Found : C, 67.70 ; H, 8.13 ; N, 3.13.

7α-epimer (24)

Rf 0.40 (EtOAc or petroleum ether-EtOAc 1:5, x 2 dev.), 0.48 (petroleum ether-EtOAc 1:1, x 10 dev.), 0.70 (CHCl3-MeOH 10:1) ; mp 166-168°C (cryst. x 4, MeOH-ether-pentane) ; $[\alpha]_D$ = 0° ± 3° (c, 0.2, CH_2Cl_2), - 15° (c, 0.2, dioxane), - 30° (c, 0.2, 95 % EtOH), - 9° (c, 0.2, CHCl3-MeOH 10:1), -10° (c, 0.3, EtOAc) ; λ max (ε) : 241-243 nm (14500) ; ν max : 3450-3300 (NH), 1735 (OCO, COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;

nmr : δ ppm 0.85 (3H, s, 18-CH3), 1.22 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 2.6 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, COOCH3), 4.3 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 5.7 (1H, s, 4-H), 6.1 (1H, d: J ∿ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 459 (M^+, 100), 428 (4), 399 (3), 345 (13), 344 (33), 328 (M^+-7-substituent, 58), 313 (58), 286 (40), 284 (16), 268 (50), 253 (33), 136 (61), 133 (72), 115 (75).

Anal. Calcd. for $C_{26}H_{37}O_6N$: C, 67.95 ; H, 8.12 ; N, 3.05.
Found : C, 67.77 ; H, 8.28 ; N, 2.90.

7β- and 7α-Hemisuccinamido-17β-hydroxy-4-androsten-3-ones, (25) and (26) :

A solution of the pure 17β-acetoxy-7β-hemisuccinamido methyl ester (21) or of its 7α-epimer (22) (0.23 g, 0.50 mmole) in 5 ml of 95 % EtOH was made alkaline with KOH (0.1 g) and was allowed to stand at room temperature overnight. The

pH of the reaction mixture was brought to 7 with HCl. The solvent was evaporated to a small volume and the pH was brought to 3-4 with HCl. The precipitate was then extracted with a CHCl3-MeOH 10:1 mixture which was washed with water and evaporated to give the acids (25) (0.17 g, 84 %) or (26) (0.18 g, 89 %), which were pure on t.l.c. These two acids were further characterized by diazomethane esterification and acetylation which gave the 17β-hydroxy-and 17β-acetoxy methyl ester precursors, (21) and (22), and (23) and (24) respectively.

7β-epimer (25)

Rf 0.21 (CHCl3--acetone-acetic acid 7:2:1), 0.57 (CHCl3-acetone-acetic acid 7:2:1, x 3 dev.) ; mp 235-240°C (cryst. x 4, aqueous MeOH) ; $[\alpha]_D$ = + 53° (c, 0.1, 95 % EtOH) ; λmax (ε) : 244 nm (14600) ; ν max (KBr) : 3600-3300 (OH, NH), 1750-1670 (COOH, NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm (d6-DMSO) 0.69 (3H, s, 18-CH3), 1.16 (3H, s, 19-CH3), 5.5 (1H, s, 4-H), 7.6 (1H, d: J ∿ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 403 (M^+, 21), 303 (7), 302 (4), 286 (M^+-7-substituent, 100), 271 (19), 268 (18), 253 (13), 136 (59), 107 (22), 83 (5).

Anal. Calcd. for $C_{23}H_{33}O_5N$: C, 68.46 ; H, 8.24 ; N, 3.47.
Found : C, 68.27 ; H, 8.27 ; N, 3.34.

7α-epimer (26)

Rf 0.22 (CHCl3-acetone-acetic acid 7:2:1), 0.62 (CHCl3-acetone-acetic acid 7:2:1, x 3 dev.) ; mp 215-218°C (cryst. x 4, aqueous EtOH) ; $[\alpha]_D$ = - 29° (c, 0.3, 95 % EtOH) ; λmax (ε) : 243 nm (13650) ; ν max (KBr) : 3600-3300 (OH, NH), 1750-1670 (COOH, NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm (d6-DMSO) 0.69 (3H, s, 18-CH3), 1.16 (3H, s, 19-CH3), 5.5 (1H, s, 4-H), 7.6 (1H, d: J ∿ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 403 (M^+, 37), 303 (5), 302 (7), 286 (M^+-7-substituent, 100), 271 (40), 268 (11), 253 (15), 136 (56), 107 (50), 83 (65).

Anal. Calcd. for $C_{23}H_{33}O_5N$: C, 68.48 ; H, 8.24 ; N, 3.47.
Found : C, 68.27 ; H, 8.27 ; N, 3.34.

17β-Tetrahydropyranyloxy-3,3'-ethylenedioxy-7-oximino-5-androstene, (28) :

A mixture of 17β-tetrahydropyranyloxy-3-ethylenedioxy-7-oxo-5-androstene (27) [9] (41.7 g, 100 mmole) and hydroxylamine hydrochloride (10.5 g, 150 mmole) was dissolved in 2 l of pyridine and stirred overnight at room temperature. The residue after evaporation to dryness was taken up in CHCl3 which was washed with water and evaporated to give the 7-oxime (28) (42.3 g, 95 %), mp 210-215°C. Rf 0.50 (petroleum ether-EtOAc 3:2) ; mp 217-220°C (cryst. x 4, CH2Cl2-MeOH) ; λmax (ε) : 238-239 nm (16000) ; ν max : 3600-3300 (N-OH), 1640 (C=N), 1100 cm^{-1} (ether) ;
nmr : δ ppm 0.81 (3H, s, 18-CH3), 1.13 (3H, s, 19-CH3), 3.6 (2H, m, OCH2 and 1H, m, 17α-H), 3.9 (4H, s, OCH2CH2O), 4.7 (1H, s, OCHO), 6.6 (1H, d: J ∿ 2 Hz, 6-H), 8.0 (1H, s broad, NOH) ;

Anal. Calcd. for $C_{26}H_{39}O_5N$: C, 70.08 ; H, 8.82 ; N, 3.14.
Found : C, 70.23 ; H, 8.82 ; N, 3.00.

7β- and 7α-Amino-17β-tetrahydropyranyloxy-3-ethylenedioxy-5-androstenes, (29) and (30) :

To a solution of 7-oxime (28) (8.9 g, 20 mmole) in 1.7 l of absolute EtOH was added 140 g of sodium during 2 h (as described before in the case of the corresponding 7-oxime-17β-acetate (2)) to give a mixture of 17β-tetrahydropyranyloxy-7-amino products (8.2 g). Preparative t.l.c. on silica gel of this crude mixture (CHCl3-MeOH-NH4OH 100:10:1, x 2 dev.) allowed the isolation of three fractions : a) the faster-moving compound (2.2 g, 25 %) was identified as the pure 5-en-7β-amine (29), b) the intermediate product (2.5 g, 29 %) containing probably the 5,6-dihydro-7β- and 7α-amines was not further examined. c) the slower-

moving compound (1.8 g, 21 %) characterized as the 5-en-7α-amine (30), had an Rf value very close to that of the intermediate spot, thus requiring several successive chromatographies in order to obtain the pure product.

7β-epimer (29)

Rf 0.55 ($CHCl_3$-MeOH-NH_4OH 100:10:1) ; mp 150-158°C (after sublimation at 200°C under 2.5 x 10^{-3} mm Hg) ;

nmr : δppm 0.78 (3H,s,18-CH_3), 1.04 (3H,s,19-CH_3), 3.1 (1H,m,7α-H), 3.6 (3H,m, OCH_2 and 17α-H), 3.9 (4H,s,OCH_2CH_2O), 4.6 (1H,s, OCHO), 5.2 (1H,s broad,6-H) ;

mass spectrum (70 eV), m/e (rel. intensity) 431 (M^+, 25), 99 (100), 85 (THP, 90).

Anal. Calcd. for $C_{26}H_{41}O_4N$: C, 72.35 ; H, 9.58 ; N, 3.25.
Found : C, 72.53 ; H, 9.65 ; N, 3.06.

7α-epimer (30)

Rf 0.37 ($CHCl_3$-MeOH-NH_4OH 100:10:1) ; mp 112-140°C (after sublimation at 200°C under 2.5 . 10^{-3} mm Hg) ;

nmr : δ ppm 0.78 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.1 (1H, m, 7β-H), 3.6 (3H, m, OCH_2 and 17α-H), 3.9 (4H, s, OCH_2CH_2O), 4.6 (1H, s, OCHO), 5.5 (1H, d: $J_{6H,7\beta H}$ ≃ 5 Hz, 6-H) ;

mass spectrum (70 eV), m/e (rel. intensity) 431 (M^+, 10), 99 (100), 85 (THP, 60).

Anal. Calcd. for $C_{26}H_{41}O_4N$: C, 72.35 ; H, 9.58 ; N, 3.25.
Found : C, 72.60 ; H, 9.69 ; N, 3.11.

7β- and 7α-Acetamido-17β-tetrahydropyranyloxy-3,3'-ethylenedioxy-5-androstenes, (31) and (32) :

A solution of the pure 5-en-7β-amine (29) or 5-en-7α-amine (30) (0.43 g, 1.0 mmole) in 10 ml of pyridine-acetic anhydride 5:1 mixture, was allowed to stand at room temperature overnight and was then evaporated under reduced pressure. The residue was purified by t.l.c. on silica gel (EtOAc, x 5 dev.) to give the 5-en-7β-acetamide (31) (0.39 g, 82 %) or 7α-acetamide (32) (0.40 g, 84 %).

7β-epimer (31)

Rf 0.34 (CH_2Cl_2-EtOAc 1:3, x 3 dev.), 0.38 (EtOAc, x 3 dev.), 0.52 ($CHCl_3$-MeOH 10:1) ; mp 220-240°C then 302-305°C (cryst. x 4, CH_2Cl_2-ether) ; ν max : 3450-3300 (NH), 1680 (NHCO), 1100 cm^{-1} (ether) ; $[\alpha]_D$ = + 49° (c, 0.2, CH_2Cl_2) ;

nmr : δ ppm 0.78 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 1.93 (3H, s, $NHCOCH_3$), 3.6 (3H, m, OCH_2 and 17α-H), 3.9 (4H, s, OCH_2CH_2O), 4.2 (1H, m, 7α-H), 4.6 (1H, s, OCHO), 5.1 (1H, t: $J_{6H,7\alpha H}$ and $J_{6H,4\beta H}$ ≃ 2 Hz, 6-H), 5.2 (1H, d: J ≃ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 473 (M^+, 10), 428 (5), 414 (M^+-7-substituent, 3), 412 (9), 389 (M^+-THP, 28), 330 (M^+-THP-7-substituent, 16), 328 (26), 302 (10), 286 (12), 182 (4), 99 (100), 85 (THP, 30).

No satisfactory elemental analysis could be obtained for this compound [3].

7α-epimer (32)

Rf 0.24 (CH_2Cl_2-EtOAc 1:3, x 3 dev.), 0.21 (EtOAc, x 3 dev.), 0.58 ($CHCl_3$-MeOH 10:1) ; mp 118-120°C → resin (cryst. x 4, ether-hexane) ; ν max : 3450-3300 (NH), 1680 (NHCO), 1100 cm^{-1} (ether) ; $[\alpha]_D$ = 148° (c, 0.3, CH_2Cl_2) ;

nmr : δ ppm 0.78 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 1.95 (3H, s, $NHCOCH_3$), 3.6 (3H, m, OCH_2 and 17α-H), 4.4 (1H, m, 7β-H), 4.6 (1H, s, OCHO), 5.2 (1H, d: J ≃ 10 Hz, NHCO), 5.3 (1H, q: $J_{6H,7\beta H}$ ≃ 5 Hz, $J_{6H,4\beta H}$ ≃ 2 Hz, 6-H) ;

mass spectrum (70 eV), m/e (rel. intensity) 473 (M^+, 6), 428 (4), 414 (M^+-7-substituent, 4), 412 (14), 389 (M^+-THP, 7), 330 (M^+-THP-7-substituent, 13), 328 (6), 302 (4), 286 (2), 182 (11), 99 (100), 85 (THP, 48).

No satisfactory elemental analysis could be obtained for this compound [3].

7β- and 7α-Terephthalamido-17β-tetrahydropyranyloxy-3,3'-ethylenedioxy-5-androstene methyl esters, (33) and (34) :

1. Preparation of the mono-esterified reagent p-ClCO C_6H_4 $COOCH_3$: to the clear solution (100 ml) of pure terephthaloyl dichloride, p-ClCO-C_6H_4COCl (≃ 4 g, 20 mmole) in 100 ml of anhydrous toluene was added 15 ml of tri-n-butylamine and the reaction mixture was cooled at 15°C and stirred vigorously. Then, anhydrous MeOH (1.25 ml, 31 mmole) was added dropwise and stirring was prolonged for 0.5 h. The use of a 0.5 fold excess of MeOH was imperative in order to transform all the dichloride. The absence of residual dichloride was systematically verified by t.l.c. on silica gel (petroleum ether-EtOAc 1:3) after condensation of the crude reagent with a small amount of 5-en-7-amine, (29) or (30). Any trace of remaining dichloride led to the formation of a slower-moving product than the expected 7-terephthalamido methyl esters, (33) and (34). The above preparation method, as well as the integration curves of the nmr spectrum of this polar compound suggest that two steroid moieties were coupled to the dichloride. The other 1,4-benzene dicarboxylic acid dimethyl ester unreactive by-product was isolated as a UV-adsorbing spot at the top of silica gel plates and was found, eventhough less than stoechiometric amounts of MeOH were employed : Rf 0.6 (petroleum ether-EtOAc 4:1) ; mp 132-136°C (lit. [14], 141-142°C subl.) ; nmr : δ ppm 3.9 (6H, s, COOCH3), 8.0 (4H, s, arom. H). On the other hand, this chromatography resulted in a poor recovery of the mono-acid chloride from silica gel, owing to rapid hydrolysis to the corresponding carboxylic acid. Therefore, the following acylations were made with the above crude reagent.

2. Acylation of the 7-amines : the pure 5-en-7β-amine (29) or 5-en-7α-amine (30) (0.43 g, 1.0 mmole), were added to 40 ml of the preceding reagent (≃ 4 mmole) and left to react at room temperature for 1 h. After evaporation of the solvent under reduced pressure, the reaction mixture was purified by t.l.c. on silica gel (petroleum ether-EtOAc 1:2) to give the pure 5-en-7β-terephthalamido methyl ester (33) (0.44 g, 74 %) or the corresponding 7α-epimer (34) (0.45 g, 76 %). As mentioned above for the 5-en-7α-hemisuccinamides, (16) or (18), the pure 5-en-7α-terephthalamido methyl ester was also isolated from 5,6-dihydro contaminants as the slower-moving spot by t.l.c. on silica gel (petroleum ether-EtOAc 3:2, x 3 dev.) of the acylation product of the crude mixture of 7-amines.

7β-epimer (33)

Rf 0.50 (petroleum ether-EtOAc 1:3), 0.40 (petroleum ether-EtOAc 3:2, x 3 dev.), 0.40 (CHCl3-EtOAc 1:1), 0.69 (CHCl3-MeOH 20:1) ; mp 220-230°C then 258-262°C (cryst. x 4, CH_2Cl_2-hexane) ; λ max (ε) : 238-243 nm (19300) ; ν max : 3450-3300 (NH), 1730 (COO), 1680 (NHCO), 1100 cm^{-1} (ether) ; $[\alpha]_D$ = + 68° (c, 0.1, CH_2Cl_2) ;

nmr : δ ppm 0.81 (3H, s, 18-CH3), 1.11 (3H, s, 19-CH3), 3.5-3.9 (4H, OCH_2, 17α-H and 7α-H), 3.9 (7H, s, OCH_2CH_2O and COOCH3), 4.6 (1H, s, OCHO), 5.2 (1H, t, J6H,7αH and J6H,4βH ≃ 2 Hz, 6-H), 5.7 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;

mass spectrum (70 eV), m/e (rel. intensity) 593 (M^+, 17), 532 (17), 509 (M^+-THP, 12), 464 (7), 448 (15), 414 (M^+-7-substituent, 14), 330 (M^+-THP-7-substituent, 19), 179 (24), 163 (75), 99 (100), 85 (THP, 85).

Anal. Calcd. for $C_{35}H_{47}O_7N$: C, 70.80 ; H, 7.98 ; N, 2.36.
Found : C, 70.68 ; H, 8.04 ; N, 2.26.

7α-epimer (34)

Rf 0.60 (petroleum ether-EtOAc 1:3), 0.56 (petroleum ether-EtOAc 3:2, x 3 dev.), 0.50 (CHCl3-EtOAc 1:1), 0.72 (CHCl3-MeOH 20:1) ; mp 130-133°C → resin (cryst. x 4, CH_2Cl_2-isopropylether) ; λmax (ε) : 237-241 nm (18000) ; νmax : 3450-3300 (NH), 1730 (COO), 1680 (NHCO), 1100 cm^{-1} (ether) ; $[\alpha]_D$ = 129° (c, 0.1, CH_2Cl_2) ;

nmr : δ ppm 0.81 (3H, s, 18-CH3), 1.10 (3H, s, 19-CH3), 3.7 (3H, m, OCH2 and 17α-H), 3.9 (7H, s broad, OCH2CH2O and COOCH3), 4.6-4.7 (2H, m, OCHO and 7β-H), 5.4 (1H, q: J6H,7βH ≃ 5 Hz and J6H,4βH ≃ 2 Hz, 6-H), 5.9 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) 593 (M^+, 1), 532 (0.5), 509 (M^+-THP, 19), 464 (8), 448 (12), 414 (M^+-7-substituent, 1), 330 (M^+-THP-7-substituent, 16), 179 (10), 163 (35), 99 (100), 85 (THP, 5).

Anal. Calcd. for $C_{35}H_{47}O_7N$: C, 70.80 ; H, 7.98 ; N, 2.36.
Found : C, 70.97 ; H, 7.87 ; N, 2.31.

7β- and 7α-Terephthalamido-17β-hydroxy-4-androsten-3-one methyl esters, (35) and (36) :

A solution of the pure 3-ethylenedioxy-5-en-7β-terephthalamido methyl ester (33) or of its 7α-epimer (34) (0.30 g, 0.5 mmole) in 30 ml of a dioxane-water 9:1 mixture was acidified to pH 2 with HCl, as described above for the corresponding hemisuccinamido methyl esters, (23) and (24). The pure 4-en-3-oxo-17β-hydroxy-7β-terephthalamido methyl ester (35) (0.19 g, 82 %), or its 7α-epimer (36) (0.20 g, 86 %) were isolated by t.l.c. on silica gel CHCl3-EtOAc 1:1, x 3 dev.). The low solubility of the pure derivatives required the use of very low concentrations of product on each t.l.c. plate in order to prevent trailing of the spots. These two products were also obtained after diazomethane esterification of the final 4-en-3-oxo-7β- and 7α-terephthalamides, (39) and (40) (vide infra).

7β-epimer (35)

Rf 0.29 (EtOAc), 0.21 (petroleum ether-EtOAc 1:3), 0.16 (CHCl3-EtOAc 1:1), 0.29 (CHCl3-MeOH 10:1) ; mp 243-245°C (cryst. x 4, CHCl3-EtOAc) ; $[\alpha]_D$ = + 19° (c, 0.3, dioxane), + 18° (c, 0.2, 95 % EtOH), + 20° (c, 0.2, CHCl3-MeOH 10:1), insoluble in CH2Cl2 ; λ max (ε) : 238-245 nm (28500) ; νmax (CHCl3) : 3650-3300 (OH, NH), 1730 (COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.82 (3H, s, 18-CH3), 1.25 (3H, s, 19-CH3), 3.6 (1H, m, 17α-H), 3.9 (3H, s, COOCH3 and 1H, m, 7α-H), 5.7 (1H, s, 4-H), 6.0 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) 465 (M^+, 7), 434 (3), 342 (1), 302 (7), 286 (M^+-7-substituent, 51), 271 (10), 268 (11), 253 (9), 242 (7), 180 (34), 163 (100), 148 (35), 136 (37).

Anal. Calcd. for $C_{28}H_{35}O_5N$: C, 72.23 ; H, 7.58 ; N, 3.01.
Found : C, 72.17 ; H, 7.47 ; N, 2.89.

7α-epimer (36)

Rf 0.42 (EtOAc), 0.25 (petroleum ether-EtOAc 1:3), 0.23 (CHCl3-EtOAc 1:1), 0.40 (CHCl3-MeOH 10:1) ; mp 257-265°C (cryst. x 4, CH2Cl2-ether) ; $[\alpha]_D$ = + 18° (c, 0.9, CH2Cl2), - 13° (c, 0.3, dioxane), - 12° (c, 0.2, 95 % EtOH), + 18° (c, 0.2, CHCl3-MeOH 10:1) ; λ max (ε) : 237-242 nm (28700) ; νmax (CHCl3) : 3650-3300 (OH, NH), 1730 (COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm 0.82 (3H, s, 18-CH3), 1.26 (3H, s, 19-CH3), 3.6 (1H, m, 17α-H), 3.9 (3H, s, COOCH3), 4.5 (1H, m, 7β-H), 5.6 (1H, s, 4-H), 6.2 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) 465 (M^+, 31), 434 (3), 342 (10), 302 (6), 286 (M^+-7-substituent, 64), 271 (22), 268 (9), 253 (8), 242 (8), 180 (8), 163 (100), 148 (15), 136 (30).

Anal. Calcd. for $C_{28}H_{35}O_5N$: C, 72.23 ; H, 7.58 ; N, 3.01
Found : C, 72.20 ; H, 7.37 ; N, 2.95.

7β- and 7α-Terephthalamido-3-oxo-4-androsten-17β-yl acetate methyl esters, (37) and (38) :

A solution of the pure 17β-hydroxy-7β-terephthalamido methyl ester (35) or of its 7α-epimer (36) (0.47 g, 10 mmole) in 10 ml of pyridine-acetic anhydride 5:1

mixture was allowed to stand at room temperature overnight and was then evaporated under reduced pressure. The residue was purified by t.l.c. on silica gel (CHCl3-EtOAc 1:1) to give the pure 17β-acetoxy-7β-and 7α-terephthalamido methyl esters, (37) (0.46 g, 90 %) and (38) (0.47 g, 92 %). As mentioned above for the 7β-hemisuccinamido methyl ester analog (21), the 4-en-3-oxo-7β-terephthalamido methyl ester (37) can be isolated from 5,6-dihydro contaminants as the slower-moving spot after t.l.c. on silica gel (petroleum ether-EtOAc 1:2, x 3 dev.) whereas this purification could not be performed with the impure 3-ethylenedioxy-5-ene precursor (33).

7β-epimer (37)

Rf 0.29 (petroleum ether-EtOAc 1:2), 0.35 (CHCl3-EtOAc 1:1), 0.40 (CHCl3-MeOH 20:1) ; mp 263-266° C (cryst. x 4, EtOAc) ; $[\alpha]_D$ = + 37° (c, 0.1, CH2Cl2), + 33° (c, 0.1, dioxane), + 32° (c, 0.2, 95 % EtOH), + 45° (c, 0.05, CHCl3-MeOH 10:1) ; λ max (ε) : 238-245 nm (27800) ; νmax (CH2Cl2) : 3450-3300 (NH), 1730 (OCO, COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm 0.86 (3H, s, 18-CH3), 1.25 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 3.9 (3H, s, COOCH3 and 1H, m, 7α-H), 4.6 (1H, m, 17α-H), 5.7 (1H, s, 4-H), 6.4 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) 507 (M^+, 8), 476 (3), 344 (5), 328 (M^+–7-substituent, 27), 313 (7), 286 (29), 268 (30), 253 (23), 180 (11), 179 (23), 163 (100), 148 (29), 145 (6), 136 (58), 135 (25), 133 (84), 103 (28).

Anal. Calcd. for $C_{30}H_{37}O_6N$: C, 70.98 ; H, 7.35 ; O, 18.91 ; N, 2.76.
Found : C, 70.91 ; H, 7.59 ; O, 18.99 ; N, 2.98.

7α-epimer (38)

Rf 0.39 (petroleum ether-EtOAc 1:2), 0.46 (CHCl3-EtOAc 1:1), 0.57 (CHCl3-MeOH 20:1) ; mp 222-224°C (cryst. x 4, CH2Cl2-ether-hexane) ; $[\alpha]_D$ = + 21° (c, 0.2, CH2Cl2), + 6° (c, 0.1, dioxane), + 12° (c, 0.3, 95 % EtOH) ; λ max (ε) : 238-242 nm (30000) ; νmax (CH2Cl2) : 3450-3300 (NH), 1730 (OCO, COO), 1680 (NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm 0.86 (3H, s, 18-CH3), 1.26 (3H, s, 19-CH3), 2.02 (3H, s, OCOCH3), 3.9 (3H, s, COOCH3), 4.4 (1H, m, 7β-H), 4.6 (1H, m, 17α-H), 5.6 (1H, s, 4-H), 6.5 (1H, d: J ≃ 10 Hz, NHCO), 7.5-8.1 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) 507 (M^+, 20), 476 (5), 344 (7), 328 (M^+-7-substituent, 26), 313 (14), 286 (12), 268 (15), 253 (9), 180 (20), 179 (4), 163 (100), 148 (5), 145 (10), 136 (18), 135 (23), 133 (27), 103 (13).

Anal. Calcd. for $C_{30}H_{37}O_6N$: C, 70.98 ; H, 7.35 ; O, 18.91 ; N, 2.76.
Found : C, 70.75 ; H, 7.35 ; O, 18.83 ; N, 2.87.

7β- and 7α-Terephthalamido-17β-hydroxy-4-androsten-3-ones, (39) and (40) :

A solution of the pure 17β-acetoxy-7β-terephthalamido methyl ester (37) or of its 7α-epimer (38) (0.25 g, 0.50 mmole) in 5 ml of 95 % EtOH was made alkaline with KOH (0.1 g) and was allowed to stand at room temperature overnight, as described above for the 7-hemisuccinamido methyl ester analogs, (21) and (22). Extraction at pH 3-4 gave the pure carboxylic acids, (39) (0.19 g, 85 %) or (40) (0.20 g, 90 %). These acids were further characterized by diazomethane esterification and acetylation which gave nearly quantitative yields of the 17β-hydroxy methyl esters, (35) and (36), and 17β-acetoxy methyl esters, (37) and (38) respectively.

7β-epimer (39)

Rf 0.42 (CHCl3-acetone-acetic acid 7:2:1), 0.13 (CHCl3-acetone-acetic acid 70:25:1) ; mp 228-235°C (dec.) (cryst. x 4, EtOH-acetone) ; $[\alpha]_D$ = + 18° (c, 0.3, 95 % EtOH) ; λ max (ε) : 238-245 nm (26000) ; νmax (KBr) : 3600-3300 (OH, NH), 1750-1670 (COOH, NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δ ppm (d6-DMSO) 0.70 (3H, s, 18-CH3), 1.20 (3H, s, 19-CH3), 3.5 (1H, m, 17α-H), 5.6 (1H, s, 4-H), 7.5-8.5 (4H, m, arom. H) ;
mass spectrum (70 eV), m/e (rel. intensity) no mass peak at 593, 286 (M^+-7-

substituent, 10), 271 (1), 269 (1), 268 (3), 253 (3), 242 (2), 227 (3), 165 (45), 149 (100), 136 (14), 121 (33), 65 (36).
No satisfactory elemental analysis could be obtained for this compound [3].

7α-epimer (40)

Rf 0.54 ($CHCl_3$--acetone-acetic acid 7:2:1), 0.19 ($CHCl_3$-acetone-acetic acid 70:25:1) ; mp 280-285°C (dec.) (cryst. x 4, MeOH) ; $[\alpha]_D$ = - 10° (c, 0.3, 95 % EtOH) ;λ max (ε) : 238-242 nm (26000) ; νmax (KBr) : 3600-3300 (OH, NH), 1750-1670 (COOH, NHCO, conj. CO), 1620 cm^{-1} (conj. C=C) ;
nmr : δppm (d6-DMSO) 0.70 (3H, s, 18-CH_3), 1.20 (3H, s,19-CH_3), 3.5 (1H, m, 17α-H), 4.3 (1H, m, 7β-H), 5.5 (1H, s, 4-H) ;
mass spectrum (70 eV), m/e (rel. intensity) 451 (M^+, 9, 286 (M^+-7-substituent, 48), 271 (12), 269 (3), 268 (10), 253 (10), 242 (7), 227 (8), 165 (33), 149 (100), 136 (42), 121 (35), 65 (45).
No satisfactory elemental analysis could be obtained for this compound [3].

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Malcolm Donaldson for his help in editing the manuscript, Mrs. Marcelle Vericel for her excellent technical help and Miss Joëlle Bois for her expert secretarial assistance. The investigation was supported by Research grants from INSERM CRL n° 73.05.083 and 77.5.209.4.

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