SYNTHESIS AND STEREOCHEMISTRY OF C-3- AND C-7-LINKED (O-CARBOXYMETHYL)-OXIMINO- AND HEMISUCCINAMIDO DERIVATIVES OF 5α-DIHYDROTESTOSTERONE

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ABSTRACT

The 3-(O-carboxymethyl)oximino derivative of 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) was prepared. Thin-layer chromatography of the corresponding methyl ester showed the presence of two *syn* (60%) and *anti* (40%) geometrical isomers of the oxime chain to the C-4 position, which were characterized by ^{13}C nmr. The 3β-hemisuccinamido-5α-androstan-17β-ol was obtained after selective saponification with potassium carbonate of the 17β-hemisuccinate group of the 3,17-dihemisuccinoylated derivative of the previously described 3β-amino-5α-androstan-17β-ol. This 3β-hemisuccinamide was purified as the corresponding methyl ester-17β-acetate and was regenerated after saponification. The 3,3'-ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate was obtained in quantitative yield by catalytic hydrogenation over 10% palladium-on-charcoal of the Δ^5-7-oxo precursor in a dioxane-ethanol mixture containing traces of pyridine. The exclusive 5α-configuration of this hydrogenated product was established from nmr data and was confirmed by the synthesis of methyl 3,3'-ethylenedioxy-7-oxo-5β-cholan-24-oate as 5β-H-reference compound. The preceding 5α-H-7-ketone was converted into the 7-(O-carboxymethyl)oximino derivative (*syn* isomer to the C-6 position, exclusively) which was esterified into the corresponding methyl ester. The selective hydrolysis of the 3-ethyleneketal group was achieved by a short treatment with a formic acid-ether 1:1 (v/v) mixture at 20°C. Saponification of the latter reaction product with ethanolic potassium hydroxide gave the 7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one derivative, which was characterized as the corresponding methyl ester. The reduction of the oxime of the 5α-H-7-ketone with sodium in ethanol or with lithium-aluminium hydride gave respectively the 7β-amine or the 7α-amine as the major product. The 7β- and 7α-configurations were established from nmr spectra of the corresponding 7-acetamido derivatives. The 7β- and 7α-hemisuccinamido derivatives were prepared from the mixture of 7β- and 7α-amines, as described above for 3-derivatives and were isolated after thin-layer chromatography of the methyl esters, followed by saponification of the corresponding 17β-acetates.

INTRODUCTION

In recent years, an increasing number of anti-steroid antisera have been prepared for the purpose of developing highly specific radioimmunoassays of steroid hormones. The experimental factors which

improve the overall specificity of these antisera are far from well known. However, it can be assumed that the unambiguous assessment of the chemical purity of the haptenic steroid part of the antigen is a preliminary requirement in order to limit the heterogeneity of the antibody response, especially in those cases involving the introduction of a carboxylic side-chain in a position different from those bearing the characteristic functional groups of the steroid hormone.

This paper deals with the synthesis and the stereochemistry of C-3 and C-7-linked (O-carboxymethyl)oximino- and hemisuccinamido derivatives of 5α-androstan-17β-ol. These compounds were synthesized in order to prepare different anti-5α-dihydrotestosterone antigens and ligands which were needed in the course of immunochemical studies of the corresponding antisera [1].

SYNTHESIS

1°. 3-O-(carboxymethyl)oximino-5α-androstan-17β-ol derivatives (Fig. 1)

The crude 3-(O-carboxymethyl)oximino-5α-androstan-17β-ol (1) was prepared by condensation of (O-carboxymethyl)hydroxylamine hemihydrochloride with 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) in pyridine solution, at room temperature. This product was esterified with diazomethane to give the methyl ester (2). Thin-layer chromatography on silica gel (with a chloroform-ethyl acetate 3:1 mixture) of this crude methyl ester showed the presence of the two *syn-anti* geometrical isomers of the oxime chain, (2a) (60%, slower-moving spot) and (2b) (40%) which were also characterized as the corresponding 17β-acetates (3a) and (3b).

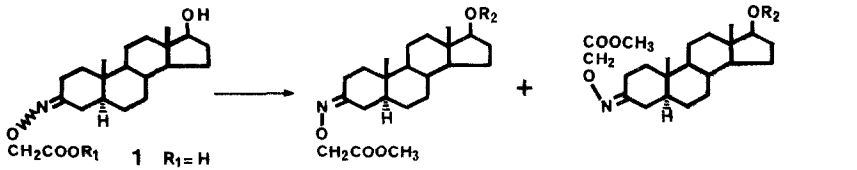


Fig. 1

The ^{1}H nmr spectra of these two isomers were identical, both showing a multiplet signal centered at 3.1 ppm attributable either to the 2α- or to the 4α-protons which are respectively deshielded by the nitrogen-linked oxygen atom, according to the *anti* or *syn* orientation of the oxime chain to the C-4 position. However, a comparison of the ^{13}C nmr signals of the α- and β- carbon atoms adjacent to the oxime group of each isolated isomer allowed the establishment of the corresponding *syn-anti* configurations. The upfield shifts of C-2 (7 ppm) and C-1 (1 ppm) carbon atoms of the minor isomer (2b) were in good agreement with previously reported values determined in the case of *syn-anti* geometrical isomers of oximes of cyclohexanone derivatives [2]. Therefore, with the assumption that (O-carboxymethyl)oxime and oxime groups exert similar effects on the chemical shifts of the adjacent carbon atoms, the comparison of the ^{13}C nmr shifts mentioned above with those reported in the preceding study allowed the assignment of a *syn* orientation (to the C-4 position) to the 3-(O-carboxymethyl)oxime group of the major isomer (2a) and of an *anti* orientation to the 3-(O-carboxymethyl)oxime group of the minor isomer (2b).

Furthermore, the higher positive value of the optical rotation of the *syn* isomer (2a) (α_D = + 23°), as compared to that observed for the *anti* isomer (2b) (α = + 2°), was consistent with previously reported results concerning the *syn* and *anti* isomers of 3-O-(carboxymethyl)oximino Δ-4 steroids [3], thus confirming the *syn-anti* assignments described above. However, in contrast to 3-(O-carboxymethyl) oximino derivatives of Δ-4 steroids which contained the *anti* isomer as the major product (55%) [3], the crude 3-(O-carboxymethyl)oximino derivative of 5α-dihydrotestosterone showed a higher percentage of *syn* isomer (60%) than of *anti* isomer (40%).

Although no noticeable *syn-anti* interconversion of 3-(O-carboxymethyl)oxime isomers was found to occur throughout the present work except after a prolonged heating, only the crude acid (1) (60:40 mixture of *syn-anti* isomers) and the corresponding crude methyl ester (2) were employed in biological studies [1].

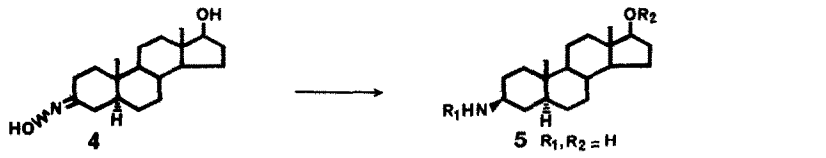
It should be mentioned that the infrared spectra of the 3-(O-carboxymethyl)oximino methyl ester derivatives (as well as those of 7-(O-carboxymethyl)oximino methyl ester derivatives described below),

recorded either in carbon tetrachloride or chloroform solution, show the presence of two peaks of nearly equal intensities at 1740 and 1765 cm^{-1}. From the analogy with previous studies concerning the infrared spectra of alicyclic α-halogenated carbonyl groups [4], it can be assumed that the 1765 cm^{-1} peak might result from dipole-dipole interactions between the carbonyl group of the ester and the oxygen atom of the oxime chain which occur in the coplanar conformations of these two groups. This characteristic feature had previously been observed in the case of methyl esters of 3- and 7-(O-carboxymethyl)oximino derivatives of Δ^4-3-oxo and Δ^5-7-oxo-steroids [3]. The presence of a double peak has also been detected in the infrared spectra of the acid precursors even though a broad peak was generally observed.

2°. 3β-Hemisuccinamido-5α-androstan-17β-ol derivatives (Fig. 2)

The reduction with sodium in n-propanol of 3-oximino-5α-androstan-17β-ol (4) (crude mixture of *syn-anti* geometrical isomers) gave, as previously reported [5], 3β-amino-5α-androstan-17β-ol (5) (∿ 90% of the isolated amino products) as the major product.

The pure 3β-amino isomer was isolated from its 3α-isomer contaminant by fractional crystallization from benzene or ethyl acetate and was further characterized as the 3β-acetamido derivative (6). The



5 $R_1, R_2 = H$

6 $R_1, R_2 = COCH_3$

7 $R_1 = CO(CH_2)_2COOH$, $R_2 = H$

8 $R_1 = CO(CH_2)_2COOCH_3$, $R_2 = H$

9 $R_1 = CO(CH_2)_2COOCH_3$, $R_2 = COCH_3$

Fig. 2

assignment of a 3β-configuration to the major amino product was in good agreement with the results of the reduction with sodium in alcohol of 3-oximino-5α-androstane which was found to give predominantly the equatorial 3β-amino isomer [6]. This stereochemistry was confirmed by

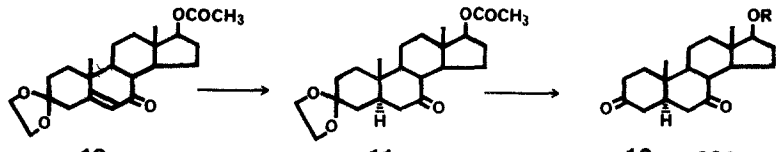
comparing the nmr spectrum of the corresponding 3β-acetamide (6) to that of the 3α-acetamide. As expected from the two studies cited above [5, 6], the methyl signal of the equatorial 3β-acetamido group was observed at higher field (1.95 ppm) than the corresponding signal of the 3α-epimer (1.98 ppm), while the 3α-proton signal appeared as a multiplet centered at 3.7 ppm instead of 4.1 ppm for the 3β-proton of the 3α-epimer. These assignments were also confirmed by the slight but characteristic downfield shift (0.79 to 0.80 ppm) of the C-19 methyl signal which was observed by comparing the 3β- to the 3α-acetamido isomer [6].

The treatment of 3β-amino-5α-androstan-17β-ol (5) with an excess of succinic anhydride in pyridine solution gave a 3,17-dihemisuccinoylated product which was not isolated. This crude product was refluxed for several hours in a methanolic solution of potassium carbonate, thus allowing the selective saponification of the 17β-hemisuccinate group to give the 3β-hemisuccinamido-5α-androstan-17β-ol (7). This acid was esterified into the methyl ester (8) with an ethereal solution of diazomethane. This methyl ester was also characterized as the corresponding 17β-acetate (9). In order to rule out the possibility of the presence of 17β-hemisuccinate contaminants in the crude acid mentioned above, the pure 3β-hemisuccinamide (7) employed in biological studies [1] was prepared by saponification of the pure 17β-acetate (9) with an ethanolic solution of potassium hydroxide. This acid was characterized again as the corresponding methyl ester (8) and acetate (9). No trace of 3β-amine (5) was detected after this saponification step.

3°. Synthesis and 5α-stereochemistry of 3,3'-ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate (11) (Fig. 3)

The different C-7-linked steroid derivatives described in this study were all synthesized from the title 5α-H-7-ketone (11). This compound was obtained by hydrogenation over a 10% palladium-on-charcoal catalyst of 3,3'-ethylenedioxy-7-oxo-androst-5-en-17β-yl acetate (10) prepared from the corresponding Δ^5-precursor according to a previously reported allylic oxidation procedure [7]. This hydrogenation was performed under atmospheric pressure in a 1:1 mixture (v/v) of dioxane and ethanol, to which traces of pyridine were added in order to prevent the hydrolysis of the 3-ethyleneketal group and to increase the amount of

5α-H-product formed [8]. The crude hydrogenation product appeared as a single homogeneous spot after thin-layer chromatography. Fractional crystallization did not allow the detection of traces of 5β-H-product in mother liquors, as observed by the absence of modifications of nmr spectra. While this study was in progress, the 7-ketone (11) was also obtained, but in 60% yield only, after catalytic hydrogenation performed in ethyl acetate [9].



10 11 12 R=COCH3 13 R=H

Fig. 3

The expected 5α-stereochemistry of the hydrogenated product (11) [8, 10] was confirmed by the presence in the nmr spectrum of the C-19 methyl signal at 1.09 ppm. This value was very close to that calculated from the additivity rules of chemical shifts [11] due to substituents at C-3, C-7 and C-17 positions of the 5α-androstane skeleton (1.10 ppm) but was significantly different from that calculated in the case of the 5β-androstane epimer (1.23 ppm).

Since the nmr signal of the C-19 methyl group was known to depend very little on the substituents in the D-ring [12], the latter calculated value (1.23 ppm) was unambiguously confirmed by the C-19 methyl chemical shift of the methyl 3,3'-ethylenedioxy-7-oxo-5β-cholan-24-oate (17) observed at 1.21 ppm, thus excluding any possibility of a 5β-configuration of the 7-ketone (11) [for the synthesis of (17), *vide infra*].

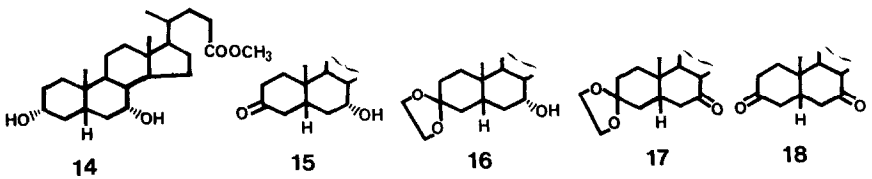
It should be mentioned that the small difference which was observed in a previous report [13] between the optical rotations of 3,3'-ethylenedioxy-5α-androstan-17β-yl acetate (α_D^{25} = + 11°) and of its 5β-isomer (α_D^{26} = + 19.9°), whereas the molecular rotation increments of a 7-oxo group are known to have similar values in both 5α- and 5β-series ($\Delta M_D \sim -230°$) [14], did not allow the use of molecular rotation incre-

ment calculations in order to establish the 5α-configuration of the 7-ketone (11).

Hydrolysis of the 3-ethyleneketal group of the 7-ketone (11) with hydrochloric acid in aqueous dioxane gave the 17β-acetoxy-3,7-diketone (12) as the major product accompanied by the 17β-hydroxy-3,7-diketone (13). The nmr spectra of these two compounds showed in both cases the C-19 methyl signal at 1.29 ppm. This value was not significantly different from the calculated C-19 methyl chemical shifts in either the 5α- (1.31 ppm) or the 5β-series (1.32 ppm) and therefore could not be employed in order to distinguish the two C-5 isomers.

4°. Synthesis of methyl 3,3'-ethylenedioxy-7-oxo-5β-cholan-24-oate (17) (Fig. 4).

The selective oxidation at the C-3 position of methyl 3α,7α-dihydroxy-5β-cholan-24-oate (methyl chenodesoxycholate) (14) with the silver carbonate-Celite reagent in boiling benzene [15] gave the mono-3-ketone (15) in a quantitative yield. This 3-ketone was converted into the 3-ethyleneketal (16). The oxidation of the 7α-hydroxyl group of (16) with the chromium trioxide-(pyridine)$_2$ complex in dichloromethane solution [16] gave the title 7-oxo compound (17) in 95% yield. The oxidation of the 3α,7α-diol (14) with the same chromium trioxide reagent gave the 3,7-diketone (18).



14 15 16 17 18

Fig. 4

5°. 7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one derivatives (Fig. 5)

The treatment of 17β-hydroxy-3,3'-ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate (11) with (O-carboxymethyl) hydroxylamine hemihydrochloride in pyridine solution at room temperature, followed by

esterification of the crude reaction product with an ethereal solution of diazomethane gave the 7-(O-carboxymethyl)oximino-3,3'-ethylenedioxy-5α-androstan-17β-yl acetate methyl ester (19). Thin-layer chromatography of this methyl ester showed the presence of a single product, presumably the *syn* geometrical isomer of the C-7 oxime chain to the C-6 position, as expected from the steric hindrance observed after examination of the Dreiding models of the other isomer. This *syn* assignment was confirmed by the presence in the nmr spectrum of a multiplet signal (one proton) centered at 3.2 ppm attributable to the adjacent 6α-proton which is deshielded by the nitrogen-linked oxygen atom of the oxime group, whereas no corresponding shift of the axial 8β-proton would be expected in the case of an *anti* configuration.

The selective cleavage of the 3,3'-ethyleneketal group without concomitent hydrolysis of the acid-labile 7-(O-carboxymethyl)-oxime group was achieved by a short treatment at 20°C with a formic acid-ether 1:1 (v/v) mixture [17]. The reaction time was carefully tested in preliminary assays. This allowed the attainment of a nearly quantitative yield of pure 7-(O-carboxymethyl)oximino-3-oxo-5α-androstan-17β-yl acetate methyl ester (20) uncontaminated by the 3,7-diketone (12), thus obviating further chromatographic purifications. The use of more classical hydrolysis reagents such as hydrochloric acid in aqueous dioxane, p-toluene-sulfonic acid in acetone or acetic acid at different concentrations and temperatures always gave mixtures of products.

The 7-(O-carboxymethyl)oxime methyl ester (20) was saponified with an ethanolic solution of potassium hydroxide and the reaction mixture was quickly extracted in very mild acidic conditions to give the pure 7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one (21), uncontaminated by the 3,7-diketone (13).

This crude acid (21) was esterified into the corresponding methyl ester (22) with an ethereal solution of diazomethane.

6°. 7β- and 7α-hemisuccinamido-17β-hydroxy-5α-androstan-3-one derivatives (Fig. 6)

The 3,3'-ethylenedioxy-7-oximino-5α-androstan-17β-yl acetate (23) was prepared by condensation of hydroxylamine hydrochloride with the 7-ketone (11) in pyridine solution, at room temperature. The reduction of this 7-oxime with sodium in ethanol, followed by hydrolysis of the 3-ethyleneketal group with hydrochloric acid in aqueous dioxane allowed the isolation, in about 50% yield, of a mixture (α_D = + 39°) of 7β- (24) and 7α-amino-17β-hydroxy-5α-androstan-3-one (25). The presence of these two isomers was established by the use of high-pressure liquid chromatography (HPLC) with the aid of the paired-ion chromatography (PIC) technique (see Experimental part). The first eluted peak, corresponding to the major isomer (∿ 60%), was attributed to the equatorial 7β-amine, as expected from previous studies [6], while the second peak was attributed to the 7α-amine (∿ 40%). Conversely, the reduction of the 7-oxime (23) with lithium-aluminium hydride in tetrahydrofuran solution, followed by the aforementioned acid hydrolysis of the 3-ethyleneketal group gave in about 80% yield, a mixture (α_D = - 28°) containing 90% of 7α-amine (25) and 10% of 7β-amine (24).

All attempts to isolate the pure 7β- and 7α-amino isomers by thin-layer chromatography or by fractional crystallization either of the free bases or of the corresponding hydrochlorides failed.

Therefore, the mixtures of 7β- and 7α-amino isomer were characterized as the corresponding 7-acetamido derivatives. The 7β-acetamide (26) (α_D = + 41°) and the 7α-acetamide (27) (α_D = - 19°) were separated by preparative thin-layer chromatography on silica gel (faster moving isomer = 7α-acetamide, with ethyl acetate). The C-7 configurations of these two isomers were confirmed by comparing the

corresponding nmr spectra. As expected from studies cited above [6], the methyl signal of the equatorial 7β-acetamide group was observed at higher field (1.93 ppm) than the corresponding signal of the 7α-isomer (2.00 ppm), while the 7α-proton showed a multiplet signal centered at 3.7 ppm instead of 4.2 ppm for the 7β-proton of the 7α-acetamide isomer. These assignments were also confirmed by the slight downfield shift (1.03 to 1.06 ppm) of the C-19 methyl signal which was observed by comparing the 7β- to the 7α-isomer [6].

The mixture of 7β- and 7α-amino isomers, obtained after reduction with sodium in ethanol or with lithium aluminium hydride was treated with an excess of succinic anhydride in pyridine solution and gave a 7,17-dihemisuccinoylated product which was not isolated. This crude product was refluxed overnight in a methanolic solution of potassium carbonate, thus allowing the selective saponification of the 17β-hemisuccinate group to give a mixture of 7β-hemisuccinamido- (28) and 7α-hemisuccinamido-17β-hydroxy-5α-androstan-3-one (29) isomers. These two compounds were indistinguishable by thin-layer chromatography.

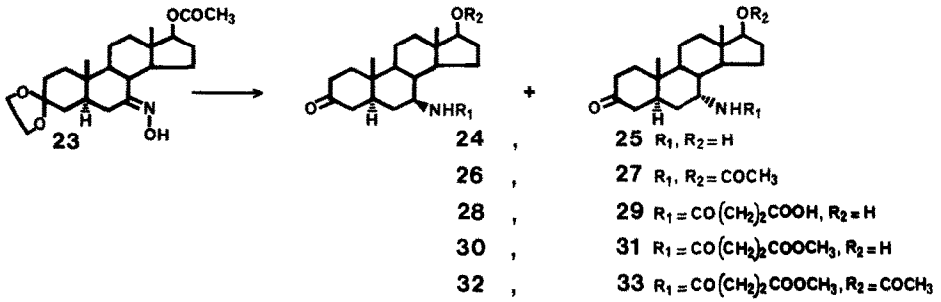


Fig. 6

The mixture of these isomeric acids was esterified into the corresponding methyl esters (30) and (31) with an ethereal solution of diazomethane. The 7β-hemisuccinamido methyl ester (30) (α_D = + 36°) and its 7α-isomer (31) (α_D = - 24°) were separated by preparative thin-layer chromatography on silica gel (faster moving isomer = 7α-hemisuccinamide, with ethyl acetate, four developments). These C-7 configurations were assigned from the analogy of the relative yields, Rf values, optical rotations and nmr spectra (signals of 7α-proton at 3.7 ppm and of C-19 methyl at 1.03 ppm for the 7β-isomer, signals of 7β-proton at 4.1 ppm

and of C-19-methyl at 1.06 ppm for the 7α-isomer) of these two isomers with those previously mentioned for the 7β- (26) and 7α-acetamides (27). These two methyl esters were also characterized as the corresponding 17β-acetates (32) (7β-isomer) and (33) (7α-isomer).

The pure 7β- and 7α-hemisuccinamido-17β-hydroxy-5α-androstan-3-one (28) and (29) employed in biological studies [1] were prepared by saponification of the 17β-acetates (32) and (33) respectively, with an ethanolic solution of potassium hydroxide. These acids were characterized again as the corresponding methyl esters (30) and (31), and acetates (32) and (33). No trace of 7β- (24) or 7α-amine (25) was detected after this saponification step.

Although the two acids (28) and (29) were almost insoluble in ether or chloroform, the addition of methanol in order to solubilize the acids must be carefully avoided before addition of diazomethane, since the presence of methanol was found to promote the formation of up to 50% of contaminants, presumably resulting from methylene insertion next to the 3-ketone. These A-homo by-products were characterized in the reaction mixture by mass spectrometry (M^+ at m/e 433 instead of 419) and infrared-spectrometry (νC=O at 1705 cm^{-1} instead of 1710 cm^{-1}). All attempts to isolate these by-products in pure form by preparative thin-layer chromatography failed.

EXPERIMENTAL

Thin-layer chromatographies were carried out on fluorescent silica gel (Merck GF 254). The petroleum ether fraction employed had a bp : 45-65°C. Melting points were taken on a Leitz hot-stage microscope and are uncorrected. 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 carbon tetrachloride solution and ^1H nmr spectra on a Varian A-60 spectrometer in deuteriochloroform solution. ^{13}C nmr spectra were obtained, using a Varian XL100 spectrometer, in deuteriochloroform solution. Mass spectra were obtained using a VG Micromass 7070 spectrometer. Elemental analyses were determined by Service Central de Microanalyse du CNRS, ESCIL, Lyon.

3-(O-carboxymethyl)oximino-5α-androstan-17β-ol (1)

A solution of 17β-hydroxy-5α-androstan-3-one (2.0 g, 6.9 mmole) and (O-carboxymethyl)hydroxylamine hemihydrochloride (2.5 g, 11.4 mmole) in 150 ml of pyridine was allowed to react at room temperature, overnight. The pyridine was evaporated under reduced pressure and the residue was extracted with chloroform. The organic extracts were washed with water and evaporated to dryness to give the crude acid (1) (2.0 g, 80%), uncontaminated with the starting 3-ketone.

Rf ∿ 0.7 (chloroform-acetone-acetic acid 7:2:1 (v/v) mixture ; the two *syn-anti* (O-carboxymethyl)oxime geometrical isomers - *vide infra* - were indistinguishable with this solvent system) ;
mp 190-200°C (crude acid) ;
$[\alpha]_D$ = + 15° (c, 0.5, ethanol) ;
νmax : 3600-3300 (OH), 1770-1740 cm^{-1} (broad peak, COOH) ;
nmr : δppm 0.72 (3H, s, 18-CH_3), 0.88 (3H, s, 19-CH_3), 3.7 (1H, m, 17α-H), 4.5 (2H, s, OCH_2).
Anal. Calcd. for $C_{21}H_{33}O_4N$: C, 69.39 ; H, 9.15 ; N, 3.85.
Found : C, 69.46 ; H, 9.26 ; N, 3.82.

After esterification into the methyl ester (2) - *vide infra* -, the crude acid (1) was found to contain 60% of *syn* (O-carboxymethyl) oxime geometrical isomer (2a) (to the C-4 position) and 40% of *anti* isomer (2b). Crystallization from chloroform was observed to increase the amount of *syn* isomer in the crystals.

3-(O-carboxymethyl)oximino-5α-androstan-17β-ol methyl ester [*syn* isomer (2a), *anti* isomer (2b)]

A solution of the crude acid (1) (1.0 g, 2.75 mmole) in 50 ml of chloroform was treated with an ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen and the organic solvents were evaporated to dryness to give the methyl ester (2) (1.0 g, 100%) ; $[\alpha]_D$ = + 15° (c, 0.7, dichloromethane).

This crude methyl ester showed two contiguous spots after thin-layer chromatography on silica gel (chloroform-ethyl acetate 3:1) corresponding to the *syn* (60%, slower-moving product) (2a) and *anti* (40%, faster-moving product) (2b) geometrical isomers to the C-4 position *(vide infra)*. Crystallization from a dichloromethane-ethyl acetate mixture was observed to increase the amount of the *syn* isomer in the crystals.

The two isomers were isolated after preparative thin-layer chromatography on silica gel (Rf ∿ 0.6, chloroform-ethyl acetate 3:1, two successive developments).

Slower-moving product : *syn* isomer to the C-4 position (2a)
Rf 0.3 (chloroform-ethyl acetate 3:1) ;
mp 145-146°C (recrystallized four times from a dichloromethane-ethyl acetate mixture) ;
$[\alpha]_D$ = + 23° (c, 0.3, dichloromethane) ;
νmax : 3610 (OH), 1765 and 1740 ($COOCH_3$), 1640 cm^{-1} (C=N) ;
^1H nmr : δppm 0.72 (3H, s, 18-CH_3), 0.87 (3H, s, 19-CH_3), 3.1 (1H, m, 4α-H), 3.7 (1H, m, 17α-H), 3.8 (3H, s, $COOCH_3$), 4.6 (2H, s, OCH_2) ;

^{13}C nmr : δppm 11.1 (C-18), 11.4 (C-19), 20.8 (C-11), 23.4 (C-15), 27.6 (C-4), 28.2 (C-2), 28.5 (C-6), 30.5 (C-6), 31.3 (C-7), 35.4 (C-8), 36.1 (C-10), 36.7 (C-12), 38.3 (C-1), 42.9 (C-13), 45.3 (C-5), 50.9 (C-14), 51.7 (OCH_3), 53.9 (C-9), 69.8 (OCH_2), 81.7 (C-17), 161.5 (C-3), 170.6 (COO) ;
mass spectrum (70 eV), m/e (rel. intensity) 377 (M^+, 70), 288 (57), 270 (15), 184 (100) ;
Anal. Calcd. for $C_{22}H_{35}O_4N$: C, 69.99 ; H, 9.34 ; N, 3.71.
Found : C, 69.90 ; H, 9.38 ; N, 3.67.

Faster-moving product : *anti* isomer (2b)
Rf 0.4 (chloroform-ethyl acetate 3:1) ;
mp 65-68°C (recrystallized four times from methanol) ;
$[\alpha]_D$ = + 2° (c, 0.5, dichloromethane) ;
νmax : 3610-3300 (OH), 1765 and 1740 ($COOCH_3$), 1640 cm^{-1} (C=N) ;
^1H nmr : δppm 0.72 (3H, s, 18-CH_3), 0.87 (3H, s, 19-CH_3), 3.1 (1H, m, (2α-H), 3.7 (1H, m, 17α-H), 3.8 (3H, s, $COOCH_3$), 4.6 (2H, s, OCH_2).
^{13}C nmr : δppm 11.1 (C-18), 11.4 (C-19), 20.8 (C-11), 21.5 (C-2), 23.4 (C-15), 28.5 (C-6), 30.5 (C-16), 31.3 (C-7), 34.1 (C-4), 35.4 (C-8), 36.1 (C-10), 36.7 (C-12), 37.4 (C-1), 42.9 (C-13), 46.6 (C-5), 50.9 (C-14), 51.7 (OCH_3), 53.9 (C-9), 69.8 (OCH_2), 81.7 (C-17), 161.8 (C-3), 170.6 (COO) ;
mass spectrum (70 eV), identical to that described for the *syn* isomer.
Anal. Calcd. for $C_{22}H_{35}O_4N$: C, 69.99 ; H, 9.34 ; N, 3.71.
Found : C, 69.93 ; H, 9.34 ; N, 3.59.

3-(O-carboxymethyl)oximino-5α-androstan-17β-yl acetate methyl ester [*syn* isomer (3a), *anti* isomer (3b)]

The crude methyl ester (2) (1.0 g, 2.65 mmole) was allowed to stand at room temperature, overnight, in the presence of 50 ml of pyridine-acetic anhydride 5:1 mixture. Evaporation to dryness under reduced pressure gave the acetate (3) (1.1 g, 100%). This residue ($[\alpha]_D^{20}$ = + 14.5°, C ∿ 1.0, dichloromethane) showed two spots after thin-layer chromatography (see the solvents below). The two products were isolated after preparative chromatography on silica gel plates (Rf ∿ 0.5, petroleum ether-ethyl acetate 3:1). These two products were also prepared by acetylation of each of the two isolated *syn* and *anti* geometrical isomers (2a) and (2b) *(vide supra)*. In these conditions no interconversion was found to occur.

Slower-moving product : *syn* isomer (60%) (3a)
Rf 0.45 (petroleum ether-ethyl acetate 3:1) ;
mp 130-132°C (recrystallized four times from methanol) ;
$[\alpha]_D$ = + 22° (c, 0.6, chloroform) ;
νmax : 1765 and 1740 cm^{-1} ($COOCH_3$) ;
nmr : δppm 0.79 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 2.04 (3H, s, $OCOCH_3$), 3.1 (1H, m, 4α-H), 3.8 (3H, s, $COOCH_3$), 4.6 (2H, s, OCH_2).
Anal. Calcd. for $C_{24}H_{37}O_5N$: C, 68.70 ; H, 8.89 ; N, 3.34.
Found : C, 68.86 ; H, 8.79 ; N, 3.51.

Faster-moving product : *anti* isomer (∿ 40%) (3b)
Rf 0.50 (petroleum ether-ethyl acetate 3:1) ;
mp 102-105°C (recrystallized four times from methanol) ;
$[\alpha]_D$ = + 2° (c, 15, chloroform) ;
νmax : 1765 and 1740 cm^{-1} ($COOCH_3$) ;
nmr : δppm 0.79 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 2.04 (3H, s, $OCOCH_3$), 3.1 (1H, m, 2α-H), 3.8 (3H, s, $COOCH_3$), 4.6 (2H, s, OCH_2).
Anal. Calcd. for $C_{24}H_{37}O_5N$: C, 68.70 ; H, 8.89 ; N, 3.34.
Found : C, 68.80 ; H, 8.94 ; N, 3.41.

3β-Amino-5α-androstan-17β-ol (5)

The title compound was prepared from 3-oximino-5α-androstan-17β-ol (4) [mp 200-208°C, α_D = + 30° (EtOH), crude mixture of *syn-anti* geometrical isomers] according to an already reported procedure [5]. To a solution of 3-oxime (4) (5.0 g, 16.4 mmole), in 1.3 l of n-propyl alcohol was added 110 g of sodium during 1 h. After 3 hrs at reflux, the solvent was evaporated under reduced pressure, water was added to the residue, and the mixture was extracted with dichloromethane. The dichloromethane layer was shaken with 0.5 l of 10% hydrochloric acid. The organic layer containing the neutral products was discarded. The aqueous layer was made alkaline with potassium hydroxide and was extracted several times with dichloromethane, which was washed with water and dried over sodium sulfate. The solvent was evaporated to dryness to give the amine (5) as a yellow gum (2.9 g, 60%). This crude product was found to contain ∿ 90% of 3β-amino isomer (5) and ∿ 10% of the 3α-isomer (percentages evaluated from the nmr spectra of the corresponding 3,17-diacetylated derivative, *vide infra*).

The pure 3β-amine (5) (controlled as above) was obtained after three successive crystallizations of the crude amine either from benzene or ethyl acetate (2.0 g, 42%).

Rf 0.2 (chloroform-methanol-NH_4OH, 100:10:1) ;
mp 155-160°C (recrystallized three times from benzene) ;
$[\alpha]_D$ = + 11° (c, 0.5, chloroform-methanol 10:1) ; lit. [5] mp 170-171°C; $[\alpha]_D$ + 12.0° ;
νmax : 3610-3300 cm^{-1} (OH and NH_2) ;
nmr : δppm 0.72 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 3.5 (1H, m, 17α-H).

3β-Acetamido-5α-androstan-17β-yl acetate (6)

A solution of crude 3β-amine (5) (1.0 g, 3.4 mmole) in 10 ml of pyridine-acetic anhydride 5:1 (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 either by fractional crystallization in a chloroform-ether mixture, or by chromatography on preparative silica gel plates (petroleum ether-ethyl acetate 1:4, three successive elutions). This resulted in a broad spot which was divided into two parts : the slower-moving compound (∿ 0.8 g) was identified as the 3β-acetamido isomer (6) (obtained also from the pure 3β-amino isomer resulting from fractional crystallization) whereas the faster-moving compound (∿ 0.1 g) was found to contain a 1:1 mixture (ratio evaluated from the nmr spectrum) of 3β- and 3α-acetamido isomers (α_D ∿ + 10°).

Further chromatographic separations did not allow the obtention of the 3α-acetamido isomer (6) in pure form.

Rf 0.3 (petroleum ether-ethyl acetate 1:4 or chloroform-ethyl acetate 1:3) ; [3α-acetamido isomer : Rf 0.35] ;
mp 257-260°C (recrystallized four times from a chloroform-ether mixture) ;
$[\alpha]_D$ = 0° ± 2° (c, 1, chloroform) ; lit. [5]: 3β-isomer, mp 284-286°C, α_D = 0° ; 3α-isomer, mp 196-197°C, α_D = + 23.5° ;
νmax : 3440, 3280, 3080 (NH), 1735 ($OCOCH_3$), 1680, 1640 cm^{-1} (NHCO) ;
nmr : δppm 0.78 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 1.95 (3H, s, $NHCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 3.7 (1H, m, 3α-H), 4.7 (1H, m, 17α-H), 5.9 (1H, d : J ∿ 8 Hz, NHCO).
[3α-acetamido isomer : 0.79 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 1.98 (3H, s, $NHCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 4.1 (1H, s, 3β-H), 4.7 (1H, m, 17α-H), 5.9 (1H, d : J ∿ 8 Hz, NHCO) ; values obtained from the nmr spectrum of a 1:1 mixture of 3β- and 3α-acetamido isomers].

3β-Hemisuccinamido-5α-androstan-17β-ol methyl ester (8)

A solution of pure 3β-amine 5 (2.0 g, 6.9 mmole) and succinic anhydride (1.5 g, 15 mmole) in 100 ml of pyridine was allowed to react at 80°C, overnight. The organic solvent was distilled off under reduced pressure and the residue was refluxed for 6 hr in 250 ml of a 2% solution of potassium carbonate in aqueous methanol in order to saponify both the excess of succinic anhydride and the 17β-hemisuccinate group. The reaction mixture was brought to pH 7 and the methanol was evaporated. The aqueous residue was acidified at pH 3-4 with conc. hydrochloric acid and extracted with a chloroform-ethanol 10:1 (v/v) mixture. The organic extracts were washed with water and evaporated to dryness. The crude acid (7) (2.1 g) was suspended in 100 ml of chloroform and esterified with an excess of ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen and the organic solvents were evaporated to dryness to give the crude methyl ester (8) (2.2 g, 79%), mp 172-180°C.

Rf 0.4 (ethyl acetate) ;
mp 195-196°C (recrystallized five times from ethyl acetate) ;
$[\alpha]_D$ = 0° ± 2° (c, 1, chloroform) ;
νmax ($CHCl_3$) : 3640-3300 (OH and NH), 1730 ($COOCH_3$), 1665 cm^{-1} (NHCO) ;
nmr : δppm 0.72 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 3.70 (3H, s, $COOCH_3$ and 2H, m, 3α-H and 17α-H), 5.7 (1H, d : J ∿ 8 Hz, NHCO) ;
mass spectrum (70 eV), m/e (relative intensity) 405 (M^+, 17), 373 (55), 314 (32), 274 (22), 256 (25), 230 (29), 215 (44), 132 (76), 100 (100).
Anal. Calcd. for $C_{24}H_{39}O_4N$: C, 71.07 ; H, 9.69 ; N, 3.45.
Found : C, 70.82 ; H, 9.69 ; N, 3.47.

3β-Hemisuccinamido-5α-androstan-17β-yl acetate methyl ester (9)

The crude 17β-hydroxy-3β-hemisuccinamido methyl ester (8) (0.5 g, 1.2 mmole) was allowed to stand overnight in the presence of 10 ml of pyridine-acetic anhydride 5:1 (v/v) mixture. Evaporation to dryness under reduced pressure gave the crude acetate (9).

Rf 0.4 (petroleum ether-ethyl acetate 1:4), 0.6 (ethyl acetate) ;
mp 173-175°C (recrystallized four times from methanol) ;
$[\alpha]_D$ = - 1° ± 2° (c, 1, dichloromethane) ;
νmax : 3400-3300 (NH), 1730 ($COOCH_3$ and $OCOCH_3$), 1665 cm^{-1} (NHCO) ;
nmr : δppm 0.78 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 2.03 (3H, s, $OCOCH_3$), 2.5 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$ and 1H, m, 3α-H), 4.7 (1H, m, 17α-H), 6.0 (1H, d : J ∿ 8 Hz, NHCO) ;
Anal. Calcd. for $C_{26}H_{41}O_5N$: C, 69.77 ; H, 9.23 ; N, 3.13.
Found : C, 69.94 ; H, 9.05 ; N, 3.14.

3β-Hemisuccinamido-5α-androstan-17β-ol (7)

A solution of the methyl ester (8) (0.5 g, 1.2 mmole) in 10 ml of 95% ethanol was made alkaline with potassium hydroxide (0.3 g) and was allowed to stand at room temperature overnight. The reaction mixture was brought to pH 7 with conc. hydrochloric acid. The ethanol was evaporated to a small volume and hydrochloric acid was added until the organic acid was totally precipitated. The product was extracted with a chloroform-ethanol 10:1 (v/v) mixture which was washed with water and evaporated to dryness to give the crude acid (7) (0.4 g, 83%, first mp ∿ 140°C, followed by partial recrystallization and a second mp ∿ 190°C).

Rf 0.6 (chloroform-acetone-acetic acid 7:2:1) ;
mp 203-205°C dec (recrystallized five times from aqueous ethanol and dried overnight at 120°C under 10^{-3} mm Hg) ;
$[\alpha]_D$ = + 1° ± 2° (c, 1, ethanol) ;
νmax ($CHCl_3$) : 3640-3300 (OH and NH), 1730 (COOH), 1660 cm^{-1} (NHCO) ;
nmr : δppm (d-6 DMSO) 0.63 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 3.5 (2H, m, 3α-H and 17α-H), 7.8 (2H, d : J ∿ 8 Hz, NHCO).
Anal. Calcd. for $C_{23}H_{37}O_4N$: C, 70.55 ; H, 9.52 ; N, 3.58.
Found : C, 70.28 ; H, 9.46 ; N, 3.46.

3,3'-Ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate (11)

A solution of 3,3'-ethylenedioxy-7-oxo-androst-5-en-17β-yl acetate (10) (4.0 g, 10.3 mmole) in 360 ml of a dioxane-95% ethanol 1:1 (v/v) mixture containing 1 ml of pyridine was hydrogenated with 3.0 g of 10% palladium-on-charcoal catalyst for 2 hours at 30°C. After filtration of the catalyst, the filtrate was evaporated to dryness to give the crude hydrogenated steroid (11) (4.0 g, 100%), mp 160-165°C, α_D = - 39° (c, 0.6, chloroform). This crude product was characterized by thin-layer chromatography *(vide infra)* which showed a single homogeneous spot at a slightly higher Rf value than that observed for the starting Δ^5-7-ketone.

Rf 0.4 (petroleum ether-ethyl acetate 3:2), 0.5 (chloroform-ethyl acetate 3:1) ;
mp 167-168°C (recrystallized four times from a dichloromethane-methanol mixture containing traces of pyridine) ;
$[\alpha]_D$ = - 43° (c, 1, chloroform) ; lit. [9] mp 176-177°C, $[\alpha]_D^{24}$ = - 45.5° ;
νmax : 1735 ($OCOCH_3$), 1710 (CO), 1090 cm^{-1} (OCH_2CH_2O) ;
nmr : δppm 0.80 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 2.05 (3H, s, $OCOCH_3$), 3.9 (4H, s, OCH_2CH_2O), 4.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74 ; H, 8.78.
Found : C, 70.62 ; H, 8.80.

3,7-dioxo-5α-androstan-17β-yl acetate (12)

A solution of the 3-ethyleneketal (11) (0.4 g, 1.0 mmole) in 10 ml of a dioxane-water 9:1 mixture (v/v) containing 1 ml of conc. hydrochloric acid was stirred for 4 hours at 20°C. The reaction mixture was neutralized with sodium bicarbonate and evaporated to dryness under reduced pressure. Water was added and the residue was extracted with chloroform. The organic layer was washed with water and evaporated to give a crude product which was purified by preparative chromatography on silica gel plates (petroleum ether-ethyl acetate 3:2). Two products were obtained, corresponding to the 17β-acetate (12) and the 17β-hydroxy steroid (13). The faster-moving product (0.25 g, 70%) was found to correspond to the title compound (12), mp 190-195°C.

Rf 0.3 (petroleum ether-ethyl acetate 3:2), 0.45 (chloroform-ethyl acetate 3:1) ;
mp 197-199°C (recrystallized four times from a dichloromethane-methanol mixture containing traces of pyridine) ;
$[\alpha]_D$ = - 36° (c, 0.7, chloroform) ;
νmax : 1735 ($OCOCH_3$), 1720-1710 cm^{-1} (7- and 3-CO) ;
nmr : δppm 0.81 (3H, s, 18-CH_3), 1.29 (3H, s, 19-CH_3), 2.07 (3H, s, $OCOCH_3$), 4.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80 ; H, 8.73.
Found : C, 72.83 ; H, 8.85.

17β-Hydroxy-5α-androstane-3,7-dione (13)

This product (13) was found to correspond to the slower-moving product in the preparative thin-layer chromatography described above (0.1 g, 30%), mp 163-168°C.

Rf 0.1 (petroleum ether-ethyl acetate 3:2), 0.5 (petroleum ether-ethyl acetate 1:3), 0.15 (chloroform-ethyl acetate 3:1) ;
mp 168-170°C (recrystallized four times from isopropyl ether) ;
$[\alpha]_D$ = - 34° (c, 0.8, chloroform) ;
νmax : 3620-3300 (OH), 1720-1710 cm^{-1} (7- and 3-CO) ;
nmr : δppm 0.77 (3H, s, 18-CH_3), 1.29 (3H, s, 19-CH_3), 3.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96 ; H, 9.27.
Found : C, 75.09 ; H, 9.20.

Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (14)

A solution of 3α,7α-dihydroxy-5β-cholan-24-oic acid ("chenodesoxycholic acid") (5.0 g, 12.3 mmole) in 50 ml of methanol was left standing overnight at 20°C, in the presence of 1.5 ml of conc. hydrochloric acid. After neutralization with sodium bicarbonate, the reaction mixture was evaporated to dryness under reduced pressure. The residue was extracted with chloroform and washed with water. The organic layer was evaporated to give the crude ester (14) as a colorless oil. All attempts to crystallize this product failed.

Rf 0.25 (petroleum ether-ethyl acetate 2:3 or chloroform-methanol 20:1) ;
$[\alpha]_D$ = + 16° (c, 0.8, chloroform) ;
νmax : 3620-3300 (OH), 1740 cm^{-1} ($COOCH_3$) ;
nmr : δppm 0.66 (3H, s, 18-CH_3), 0.92 (3H, d : J ∿ 6 Hz, 21-CH_3), 0.89 (3H, s, 19-CH_3), 3.7 (3H, s, $COOCH_3$), 3.9 (2H, m, 3β- and 7β-H).

Methyl-7α-hydroxy-3-oxo-5β-cholan-24-oate (15)

A solution of the diol (14) (1.0 g, 2.5 mmole) in 50 ml of benzene was added to a suspension of 20 g of silver carbonate-Celite reagent [15] in 250 ml of benzene. The reaction mixture was refluxed for 6 hours and turned to a black colour. After filtration through Celite, the benzenic solution was evaporated and gave the pure 3-ketone (15) as a light yellow oil (1.0 g) which was recrystallized from ether.

Rf 0.6 (petroleum ether-ethyl acetate 2:3), 0.5 (petroleum ether-ethyl acetate 2:1, 6 successive developments) ;
mp 114-116°C (recrystallized four times from ether) ;
$[\alpha]_D$ = + 17° (c, 0.8, chloroform) ; lit. [18] mp 124-125°C, $[\alpha]_D^{15}$ = + 15.2° ;
νmax : 3620-3300 (OH), 1740 ($COOCH_3$), 1715 cm^{-1} (CO) ;
nmr : δppm 0.71 (3H, s, 18-CH_3), 0.92 (3H, d : J ∿ 6 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 3.7 (3H, s, $COOCH_3$), 3.9 (1H, m, 7β-H).
Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.22 ; H, 9.97.
Found : C, 74.46 ; H, 9.90.

Methyl-3,3'-ethylenedioxy-7α-hydroxy-5β-cholan-24-oate (16)

A solution of crude 3-ketone (15) (1.0 g, 2.5 mmole) and p-toluene-sulfonic acid (80 mg) in 120 ml of anhydrous benzene containing 16 ml of ethylene glycol was stirred and refluxed under a Dean-Stark water separator for 7 hours. The mixture was washed with 5% aqueous sodium bicarbonate, then with water and evaporated under reduced pressure to give an oily residue (1.1 g) which was found to crystallize from di-isopropyl ether.

Rf 0.6 (petroleum ether-ethyl acetate 2:3), 0.5 (petroleum ether-ethyl acetate 4:1, 6 successive developments) ; this ethyleneketal (16) was indistinguishable by these chromatographies from the starting 3-ketone (15) ;
mp 124-126°C (recrystallized four times from di-isopropyl ether) ;
$[\alpha]_D$ = + 15° (c, 0.2, chloroform) ; lit. [19] mp 114-116°C ;
νmax : 3620-3300 (OH), 1740 ($COOCH_3$), 1100 cm^{-1} (OCH_2CH_2O) ;
nmr : δppm 0.66 (3H, s, 18-CH_3) ; 0.92 (3H, d, J ∿ 6 Hz, 21-CH_3), 0.93 (3H, s, 19-CH_3), 3.7 (3H, s, $COOCH_3$), 3.9 (1H, m, 7β-H), 4.00 (4H, s, OCH_2CH_2O).
Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.28 ; H, 9.89.
Found : C, 72.33 ; H, 9.72.

Methyl-3,3'-ethylenedioxy-7-oxo-5β-cholan-24-oate (17)

Anhydrous chromium trioxide (1.5 g, 15 mmole) was added to a stirred solution of dry pyridine (2.4 g, 30 mmole) in 38 ml of anhydrous dichloromethane, under a dry nitrogen atmosphere [16]. The stirring was maintained for 15 mn at 20°C. Then, a solution of the 7α-hydroxysteroid (16) (1.0 g, 2.25 mmole) in 10 ml of dichloromethane was added in one portion. A tarry-black deposit separated immediately. After stirring for 15 mn at 20°C, the reaction mixture was filtered through a small column of Florisil which retained all the brown pigments. The colorless filtrate was evaporated under reduced pressure to give the pure 7-ketone (17) as white crystals (0.95 g, 95%), mp 160-165°C.

Rf 0.7 (petroleum ether-ethyl acetate 2:1) ;
mp 168-169°C (recrystallized four times from a dichloromethane-methanol mixture containing traces of pyridine) ;
$[\alpha]_D$ = - 19° (c, 0.3, chloroform) ;
νmax : 1735 ($COOCH_3$), 1710 (CO), 1100 cm^{-1} (OCH_2CH_2O) ;
nmr : δppm 0.66 (3H, s, 18-CH_3), 0.92 (3H, d : J ∿ 6 Hz, 21-CH_3), 1,21 (3H, s, 19-CH_3), 3.7 (3H, s, $COOCH_3$), 4.00 (3H, s, OCH_2CH_2O).
Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61 ; H, 9.48.
Found : C, 72.76 ; H, 9.62.

Methyl 3,7-dioxo-5β-cholan-24-oate (18)

Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (14) was oxidized with the chromium trioxide-(pyridine)$_2$ complex in dichloromethane solution, as described above, to give the 3,7-diketone (18) in nearly quantitative yield.

Rf 0.8 (petroleum ether-ethyl acetate 2:3) ;
mp 152-155°C (recrystallized four times from a dichloromethane-methanol mixture) ;
$[\alpha]_D$ = - 36° (c, 0.2, chloroform) ;
νmax : 1740 ($COOCH_3$), 1715 cm^{-1} (CO) ;
nmr : δppm 0.70 (3H, s, 18-CH_3), 0.92 (d : J ∿ 6 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 3.7 (3H, s, $COOCH_3$).
Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59 ; H, 9.51.
Found : C, 74.46 ; H, 9.78.

7-(O-carboxymethyl)oximino-3,3'-ethylenedioxy-5α-androstan-17β-yl acetate methyl ester (19)

A solution of 3,3'-ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate (11) (3.9 g, 10 mmole) and (O-carboxymethyl)hydroxylamine hemihydrochloride (5.0 g, 22.8 mmole) in 300 ml of pyridine was allowed to react at room temperature overnight. The pyridine was evaporated under reduced pressure and the residue was extracted with chloroform. The organic extracts were washed with water and evaporated to dryness to give the crude acid (∿ 4 g) (Rf 0.25, chloroform-acetone-acetic acid 70:25:1 ; mp 218-230°C ; α_D = - 46° (C ∿ 7, chloroform). The acid was suspended in 150 ml of chloroform and was treated with an ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen and the organic solvents were evaporated to dryness to give the methyl ester (19) (4.0 g, 84%), mp 201-204°C.

Rf 0.65 (petroleum ether-ethyl acetate 1:1) ;
mp 202-203°C (recrystallized four times from methanol containing traces of pyridine) ;
$[\alpha]_D$ = - 42° (c, 0.6, chloroform) ;
νmax : 1765-1735 cm^{-1} ($COOCH_3$ and $OCOCH_3$) ;
nmr : δppm 0.80 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 2.04 (3H, s, $OCOCH_3$), 3.2 (1H, d : J ∿ 10 Hz, 6α-H), 3.8 (3H, s, $COOCH_3$), 4.0 (3H, s, OCH_2CH_2O), 4.6 (2H, s, OCH_2), 4.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{26}H_{39}O_7N$: C, 65.38 ; H, 8.23 ; N, 2.93.
Found : C, 65.55 ; H, 8.24 ; N, 2.84.

7-(O-carboxymethyl)oximino-3-oxo-5α-androstan-17β-yl acetate methyl ester (20)

The crude 7-(O-carboxymethyl)oximino-3,3'-ethylenedioxy-5α-androstan-17β-yl acetate methyl ester (19) (3.0 g, 6.3 mmole) was dissolved in 500 ml of ether and stirred for 4 mn with 500 ml of formic acid at 20°C [17]. The acid was neutralized with an excess of sodium bicarbonate and the ether layer was decanted and washed with water. The ether layer was dried over sodium sulfate and evaporated to give the methyl ester (20) (2.5 g, 90%). Thin-layer chromatography of the crude products *(vide infra)* showed the absence of any trace of untransformed starting product (19) (Rf 0.65) or of 3,7-diketone (12) (Rf 0.55). However, these contaminants were respectively observed in those cases where too short or too long reaction times were employed, and therefore should be removed by preparative thin-layer chromatography.

Rf 0.60 (petroleum ether-ethyl acetate 1:1) ;
mp 137-139°C (recrystallized four times from dichloromethane-ether) ;
$[\alpha]_D$ = - 44° (c, 0.5, chloroform) ;
νmax : 1765-1735 ($COOCH_3$ and $OCOCH_3$), 1720 cm^{-1} (CO) ;
nmr : δppm 0.82 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.04 (3H, s, $OCOCH_3$), 3.2 (1H, d : J ∿ 10 Hz, 6α-H), 3.8 (3H, s, $COOCH_3$), 4.6 (2H, s, OCH_2), 4.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{24}H_{35}O_6N$: C, 66.49 ; H, 8.14 ; N, 3.23.
Found : C, 66.34 ; H, 8.02 ; N, 3.22.

7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one (21)

A solution of the methyl ester acetate (20) (0.5 g, 1.15 mmole) in 10 ml of 95% ethanol was made alkaline with potassium hydroxide (0.3 g) and was allowed to stand at room temperature overnight. The reaction mixture was brought to pH 7 with conc. hydrochloric acid, the ethanol was evaporated to a small volume and ice-cooled 5% hydrochloric was added until pH 3 was obtained. The organic acid was extracted with chloroform, which was washed with cold water and evaporated under reduced pressure at room temperature. These very mild acidic conditions employed for this extraction allowed the obtention of the crude acid (21) (0.3 g, 69%) uncontaminated with the 3,7-diketone (13).

Rf 0.5 (chloroform-acetone-acetic acid 7:2:1 (v/v) mixture) ;
mp 191-194°C (recrystallized four times from ethyl acetate) ;
$[\alpha]_D$ = - 64° (c, 0.1, ethanol) ;
νmax ($CHCl_3$) : 3600-3300 (OH), 1765-1740-1710 cm^{-1} (broad peak, COOH and 3-CO) ;
nmr : δppm 0.77 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 3.2 (1H, d : J ∿ 10 Hz, 6α-H), 3.7 (1H, m, 17α-H), 4.7 (2H, s, OCH_2) ;
Anal. Calcd. for $C_{21}H_{31}O_5N$: C, 66.82 ; H, 8.28 ; N, 3.71.
Found : C, 66.99 ; H, 8.52 ; N, 3.51.

7-(O-carboxymethyl)oximino-17β-hydroxy-5α-androstan-3-one methyl ester (22)

A suspension of the crude acid (21) (0.5 g, 1.32 mmole) in 10 ml of chloroform was treated with an ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen and the organic solvents were evaporated to dryness to give the methyl ester (22) (0.52 g, 100%), mp 100-106°C.

Rf 0.3 (petroleum ether-ethyl acetate 1:1) ;
mp 125-127°C (recrystallized four times from dichloromethane-ether) ;
$[\alpha]_D$ = - 60° (c, 0.2, chloroform) ;
νmax : 3610 (OH), 1765 and 1745 ($COOCH_3$), 1720 cm^{-1} (CO) ;
nmr : δppm 0.77 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 3.2 (1H, d : J ∿ 10 Hz, 6α-H), 3.8 (3H, s, $COOCH_3$ and 1H, m, 17α-H), 4.6 (2H, s, OCH_2) ;
mass spectrum (70 eV), m/e (relative intensity) 391 (M^+, 100), 302 (62), 284 (31), 204 (54), 178 (32).
Anal. Calcd. for $C_{22}H_{33}O_5N$: C, 67.49 ; H, 8.50 ; N, 3.58.
Found : C, 67.32 ; H, 8.65 ; N, 3.32.

3,3'-Ethylenedioxy-7-oximino-5α-androstan-17β-yl-acetate (23)

A mixture of 3,3'-ethylenedioxy-7-oxo-5α-androstan-17β-yl acetate (11) (3.9 g, 10 mmole) and hydroxylamine hydrochloride (1.05 g, 15 mmole) was dissolved in 250 ml of pyridine and left overnight at room temperature. The residue after evaporation to dryness was taken up in chloroform which was washed with water and evaporated to give the 7-oxime (23) (3.85 g, 95%), mp 198-204°C.

Rf 0.4 (petroleum ether-ethyl acetate 3:2, Rf slightly higher than that of the 7-ketone) ;
mp 202-204°C (recrystallized four times from a dichloromethane-methanol mixture containing traces of pyridine) ;
$[\alpha]_D$ = - 105° (c, 0.9, dichloromethane) ;
νmax : 3600-3250 (N-OH), 1735 ($OCOCH_3$), 1100 cm^{-1} (OCH_2CH_2O) ;
nmr : δppm 0.82 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 2.07 (3H, s, $OCOCH_3$), 3.2 (1H, d : J ∿ 10 Hz, 6α-H). 4.0 (4H, s, OCH_2CH_2O), 4.7 (1H, m, 17α-H).
Anal. Calcd. for $C_{23}H_{35}O_5N$: C, 68.12 ; H, 8.70 ; N, 3.45.
Found : C, 67.93 ; H, 8.65 ; N, 3.39.

7β- and 7α-Amino-17β-hydroxy-5α-androstan-3-one (24) and (25)

a) Reduction with sodium in ethanol :

To a solution of 7-oxime (23) (5.5 g, 13.6 mmole) in 1.20 l of ethanol was added 95 g of sodium during 1 h. The mixture was refluxed for 2 hr, 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 150 ml of a dioxane-water 9:1 mixture (v/v) and acidified at pH 2 with conc. hydrochloric acid. The reaction mixture was refluxed for 1 hr, brought to pH 4 with sodium bicarbonate, evaporated to dryness and diluted with 0.5 l of water. A preliminary extraction with dichloromethane removed the neutral products. Then, the aqueous layer was made alkaline with sodium hydroxide and was extracted a second time with dichloromethane, which was washed three times with water. The organic solvent was evaporated to dryness to give the crude amine as a yellow gum (2.1 g, 50%).

The presence in the reaction product of two 7β- and 7α-amino-DHT isomers was established by the use of high-pressure liquid chromatography (HPLC) with the aid of the paired-ion chromatography (PIC) technique. The analytical separation of the two isomers was performed on a μBONDAPAK C18 column (Waters Associates) with a 50% aqueous methanol solution containing 0.005 M of 1-heptane-sulfonic acid (PIC B-7 reagent,

Waters Ass.). The first eluted peak (K' = 1.4) was found to correspond to the 7β-amine (24) (∿ 60% major product) while the second peak (K' = 1.8) was attributed to the 7α-amine (25) (∿ 40%) (capacity factor $K' = \frac{VR-VO}{VO}$, where VR = retention volume and VO = void volume).

All attempts to isolate the major 7β-amino-isomer by fractional crystallization of either the free base (under a nitrogen atmosphere) or the corresponding hydrochloride failed to give pure crystalline compounds, although the proportion of 7β-amino isomer was often found to be increased in the amorphous precipitates which were obtained instead of crystals.

Characteristics of the crude 7-amine :

Rf 0.3 (chloroform-methanol-NH_4OH 100:10:1 mixture, two successive developments); further developments did not allow the separation of the two 7β- and 7α-amino isomers ;

$[\alpha]_D$ = + 39° (c, 0.4, chloroform-methanol 10:1) ;

νmax (CH_2Cl_2) : 3600-3400 (OH and NH_2) ; 1705 cm^{-1} (CO) ;

nmr : δppm 0.77 (3H, s, 18-CH_3, minor 7α-amino isomer), 0.79 (3H, s, 18-CH_3, major 7β-amino isomer), 1.04 (3H, s, 19-CH_3 identical for the two 7-isomers), 3.0 (1H, broad peak, 7β-H of minor 7α-amino isomer), 3.7 (1H, m, 17α-H).

b) Reduction with lithium-aluminium hydride :

The 7α-amine (25) was also prepared as the major product of reduction of the 7-oxime (23) (3 g, 7.4 mmole) with an excess of lithium-aluminium hydride (5 g) in 150 ml of refluxing tetrahydrofuran for 12 hr, followed by the hydrolysis of the 3-ethyleneketal group with conc. hydrochloric acid (1 ml) in a dioxane-water 9:1 mixture (10 ml). The reaction mixture was extracted as described above to give (1.8 g, 80%) of crude amine, containing 90% of 7α-amine (25) and 10% of 7β-amine (24) (identified by HPLC). All attempts to purify the 7α-amine either as a free base or as hydrochloride by fractional crystallization failed. Only amorphous precipitates were obtained.

$[\alpha]_D$ = - 28° (c, 0.3, chloroform-methanol 10:1) ;

nmr : see above the characteristics of the crude 7-amine mixture.

7β- and 7α-Acetamido-3-oxo-5α-androstan-17β-yl acetate (26) and (27)

A solution of the crude 7-amino isomers (24) and (25), resulting from reduction with sodium in alcohol *(vide supra)* (2.0 g, 6.5 mmole), in 20 ml of pyridine-acetic anhydride 5:1 (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 thin-layer chromatography on silica gel (ethyl acetate, four successive developments) and gave two major products : the slower-moving compound (1.3 g, 50%) was identified as the 7β-acetamido isomer (26) whereas the faster-moving compound was the 7α-acetamido isomer (27) (0.6, 24%).

7β-Acetamido isomer (26) :

Rf 0.5 (ethyl acetate, four successive developments) ;

mp 229-232°C (recrystallized four times from a dichloromethane-ether mixture) ;

$[\alpha]_D$ = + 41° (c, 0.6, dichloromethane) ;

νmax : 3450-3300 (NH), 1735 ($OCOCH_3$), 1720 (3-CO), 1680 cm^{-1} (NHCO) ;

nmr : δppm 0.82 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.93 (3H, s, 7β-$NHCOCH_3$), 2.05 (17β-$OCOCH_3$), 3.7 (1H, m broad, 7α-H), 4.7 (1H, m, 17α-H), 5.7 (1H, d : J ∿ 10 Hz, NHCO).

Anal. Calcd. for $C_{23}H_{35}O_4N$: C, 70.92 ; H, 9.06 ; N, 3.60.
Found : C, 70.77 ; H, 8.97 ; N, 3.69.

7α-Acetamido isomer (27) :

This compound was also obtained after acetylation of the product of reduction of (23) with lithium-aluminium hydride.

Rf 0.6 (ethyl acetate, four successive developments) ;
mp 224-226°C (recrystallized four times from methanol) ;
$[\alpha]_D$ = - 19° (c, 0.7, dichloromethane) ;
νmax : 3450-3300 (NH), 1735 ($OCOCH_3$), 1720 (3-CO), 1680 cm^{-1} (NHCO) ;
nmr : δppm 0.83 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.00 (3H, s, 7α-$NHCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 4.2 (1H, m, 7β-H), 4.7 (1H, m, 17α-H), 6.3 (1H, d : J ∿ 10 Hz, NHCO).

Anal. Calcd. for $C_{23}H_{35}O_4N$: C, 70.92 ; H, 9.06 ; N, 3.60.
Found : C, 70.68 ; H, 8.84 ; N, 3.77.

7β- and 7α-Hemisuccinamido-17β-hydroxy-5α-androstan-3-one methyl ester (30) and (31)

A solution of the crude 7-amino isomers (24) and (25) resulting from sodium in alcohol reduction (2.0 g, 6.5 mmole) and succinic anhydride (1.5 g, 15 mmole) in 100 ml of pyridine was allowed to react at 80°C, overnight. The organic solvent was distilled off under reduced pressure and the residue was refluxed overnight in 850 ml of a 2% solution of potassium carbonate in aqueous methanol in order to saponify both the excess of succinic anhydride and the 17β-hemisuccinate group. The reaction mixture was brought to pH 7 and the methanol was evaporated. The aqueous residue was acidified at pH 3-4 with conc. hydrochloric acid and extracted with a chloroform-ethanol 10:1 (v/v) mixture. The organic extracts were washed with water, and evaporated to dryness. The crude acid (∿ 2.5 g) was suspended in 100 ml of chloroform and esterified with an ethereal solution of diazomethane. The excess of diazomethane was removed under a stream of nitrogen and the organic solvents were evaporated to dryness to give a mixture of isomeric methyl esters (30) and (31) (2.5 g).

7β-Hemisuccinamido isomer (30) :

Rf 0.4 (ethyl acetate, four successive developments) ;
mp 205-206°C (recrystallized four times from dichloromethane-ether) ;
$[\alpha]_D$ = + 36° (c, 0.8, chloroform) ;
νmax (CH_2Cl_2) : 3600-3300 (OH and NH), 1735 ($COOCH_3$), 1710 (3-CO), 1670 cm^{-1} (NHCO) ;
nmr : δppm 0.76 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$ and 2H, m, 7α-H and 17α-H), 5.8 (1H, d : J ∿ 10 Hz, NHCO) ;
mass spectrum (70 eV), m/e (rel. intensity) 419 (M^+, 1), 288 (100), 270 (15), 255 (23), 244 (17), 132 (57), 115 (24).

Anal. Calcd. for $C_{24}H_{37}O_5N$: C, 68.71 ; H, 8.89 ; N, 3.34.
Found : C, 68.86 ; H, 8.66 ; N, 3.30.

7α-Hemisuccinamido isomer (31) :

Rf 0.45 (ethyl acetate, four successive developments) ;

mp 184-185°C (recrystallized five times from acetone ; this sharp mp was obtained after drying the analytical sample for 6 hr at 140°C under ∿ 10^{-3} mm Hg - a partial sublimation of the product was observed - ; less severe drying conditions resulted in a presumably solvated product showing a first mp around 100°C followed by partial recrystallization and a second mp around 150°C) ;

$[\alpha]_D$ = - 24° (c, 0.3, chloroform) ;

νmax (CH_2Cl_2) : 3600-3300 (OH and NH), 1735 ($COOCH_3$), 1710 (3-CO), 1670 cm^{-1} (NHCO) ;

nmr : δppm 0.77 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$ and 1H, m, 17α-H), 4.1 (1H, m, 7β-H), 6.2 (1H, d : J ∿ 10 Hz, NHCO) ;

mass spectrum (70 eV), m/e (rel. intensity) 419 (M^+, 25), 288 (43), 270 (7), 255 (7), 244 (3), 132 (91), 115 (100).

Anal. Calcd. for $C_{24}H_{37}O_5N$: C, 68.71 ; H, 8.89 ; N, 3.34.
Found : C, 68.56 ; H, 8.77 ; N, 3.14.

7β- and 7α-Hemisuccinamido-3-oxo-5α-androstan-17β-yl acetate methyl ester (32) and (33)

Each of the two isolated 17β-hydroxy-7-hemisuccinamido methyl esters (30) and (31) was allowed to stand at room temperature, overnight, in the presence of 50 ml of pyridine acetic anhydride 5:1 (v/v) mixture. Evaporation to dryness under reduced pressure gave a quantitative yield of the corresponding crude 17β-acetates (32) and (33).

7β-Hemisuccinamido isomer (32) :

Rf 0.4 (ethyl acetate, one development, or petroleum ether-ethyl acetate 2:3, four successive developments) ;

mp 156-157°C (recrystallized four times from a dichloromethane-ether mixture) ;

$[\alpha]_D$ = + 38° (c, 0.5, dichloromethane) ;

νmax : 3450-3300 (NH), 1735 ($OCOCH_3$ and $COOCH_3$), 1720 (3-CO), 1680 cm^{-1} (NHCO) ;

nmr : δppm 0.83 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.06 (3H, s, $OCOCH_3$), 2.5 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$ and 1H, m, 7α-H), 4.7 (1H, m, 17α-H), 5.8 (1H, d : J ∿ 10 Hz, NHCO).

Anal. Calcd. for $C_{26}H_{39}O_6N$: C, 67.65 ; H, 8.52 ; N, 3.03.
Found : C, 67.36 ; H, 8.56 ; N, 2.78.

7α-Hemisuccinamido isomer (33) :

Rf 0.5 (ethyl acetate, one development, or petroleum ether-ethyl acetate 2:3, four successive developments) ;

mp 121-124°C (crystallized three times from a dichloromethane-ether-methanol mixture) ;

$[\alpha]_D$ = - 15° (c, 0.2, dichloromethane) ;

νmax : 3450-3300 (NH), 1735 ($OCOCH_3$ and $COOCH_3$), 1720 (3-CO), 1680 cm^{-1} (NHCO) ;

nmr : δppm 0.83 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.06 (3H, s, $OCOCH_3$), 2.5 (4H, m, $COCH_2CH_2CO$), 3.7 (3H, s, $COOCH_3$), 4.2 (1H, m, 7β-H), 4.7 (1H, m, 17α-H), 6.4 (1H, d : J ∿ 10 Hz, NHCO).

Anal. Calcd. for $C_{26}H_{39}O_6N$: C, 67.65 ; H, 8.52 ; N, 3.03.
Found : C, 67.48 ; H, 8.76 ; N, 3.02.

7β- and 7α-Hemisuccinamido-17β-hydroxy-5α-androstan-3-one (28) and (29)

A solution of the methyl ester acetate (32) or (33) (0.5 g, 1.1 mmole) in 10 ml of 95% ethanol was made alkaline with potassium hydroxide (0.3 g) and was allowed to stand at room temperature, overnight. The reaction mixture was brought to pH 7 with conc. hydrochloric acid. The ethanol was evaporated to a small volume, and hydrochloric acid was added until the organic acid was totally precipitated. The product was extracted with a chloroform-ethanol 10:1 (v/v) mixture which was washed with water and evaporated to dryness to give the crude acid (28) or (29) (0.4 g, 90%).

7β-Hemicuccinamido isomer (28) :

Rf 0.4 (chloroform-acetone-acetic acid 7:2:1 mixture) ;
mp 252-255°C dec (recrystallized four times from aqueous ethanol) ;
$[\alpha]_D$ = + 34° (c, 0.7, ethanol) ;
νmax (KBr) : 3600-3300 (OH and NH), 1750-1700 (broad peak, COOH and 3-CO), 1650 cm^{-1} (NHCO) ;
nmr : δppm (d6-DMSO) 0.65 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 7.5 (1H, d : J ∿ 10 Hz, NHCO).
Anal. Calcd. for $C_{23}H_{35}O_5N$: C, 68.12 ; H, 8.70 ; N, 3.45.
Found : C, 68.26 ; H, 8.65 ; N, 3.43.

7α-Hemisuccinamido isomer (29) :

Rf 0.4 (chloroform-acetone-acetic acid 7:2:1, indistinguishable from the 7β-isomer) ;
mp 237-240°C dec (recrystallized four times from aqueous ethanol) ;
$[\alpha]_D$ = - 33° (c, 0.3, ethanol) ;
νmax (KBr) : 3600-3300 (OH and NH), 1750-1700 (broad peak, COOH and 3-CO), 1650 cm^{-1} (NHCO) ;
nmr : δppm (d6-DMSO) 0.65 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.5 (4H, m, $COCH_2CH_2CO$), 7.5 (1H, d : J ∿ 10 Hz, NHCO).
Anal. Calcd. for $C_{23}H_{35}O_5N$: C, 68.12 ; H, 8.70 ; N, 3.45.
Found : C, 68.00 ; H, 8.59 ; N, 3.52.

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