A NEW CLASS OF LONG-ACTING HORMONAL STEROID PREPARATION: SYNTHESIS OF DIMERIC ETHYNODIOL AND NORTESTOSTERONE, OF DIMERIC AND TRIMERIC ANDROGENS AND OF SOME DIMERIC COMBINATIONS OF STEROIDS

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received: 8/20/74

ABSTRACT

Dimeric steroid esters containing an estradiol molecule coupled with a molecule of ethynodiol, cortexolone, or ethinylandrostenediol respectively, were synthesized. The same method was also used for dimerization of ethynodiol, nortestosterone, and testosterone, and for the preparation of a trimeric androgen consisting of two molecules testosterone linked by one molecule 5-androstene-3ß,17ß-diol. The method is based upon the direct esterification of steroid alcohols with steroid hemisuccinates in the presence of N,N'-carbonyldiimidazole. The results of preliminary experiments concerning the biological activity of some of these compounds showed a considerable prolongation of hormonal effect as compared to the respective monomer.

INTRODUCTION

Two or more estradiol molecules can be coupled to give dimeric, trimeric, or tetrameric estradiol esters. This new class of hormonal steroid preparation has been found to have a considerably longer period of effectiveness as compared to the respective monomeric steroid ester.

Consequently, it was investigated whether or not the method described in a previous report (1) would also be suitable for the synthesis of other steroid oligomeres. When the period of effectiveness of estradiol oligomeres was tested in oophorectomized rats, it became apparent that the depot-effect seemed to be due to the step-wise release of steroid molecules from the short steroid chains. The protracted degradation of the steroid oligomeres seemed to result in a remarkably even rate of release of active hormone from primary or secondary depots. These features seemed to merit the preparation of other steroid oligomeres which could be tested for their possible application as therapeutic agents or long-acting injectables in humans. Therefore the synthesis of dimeric and trimeric androgen and progestogen derivatives with a depot-effect was undertaken.

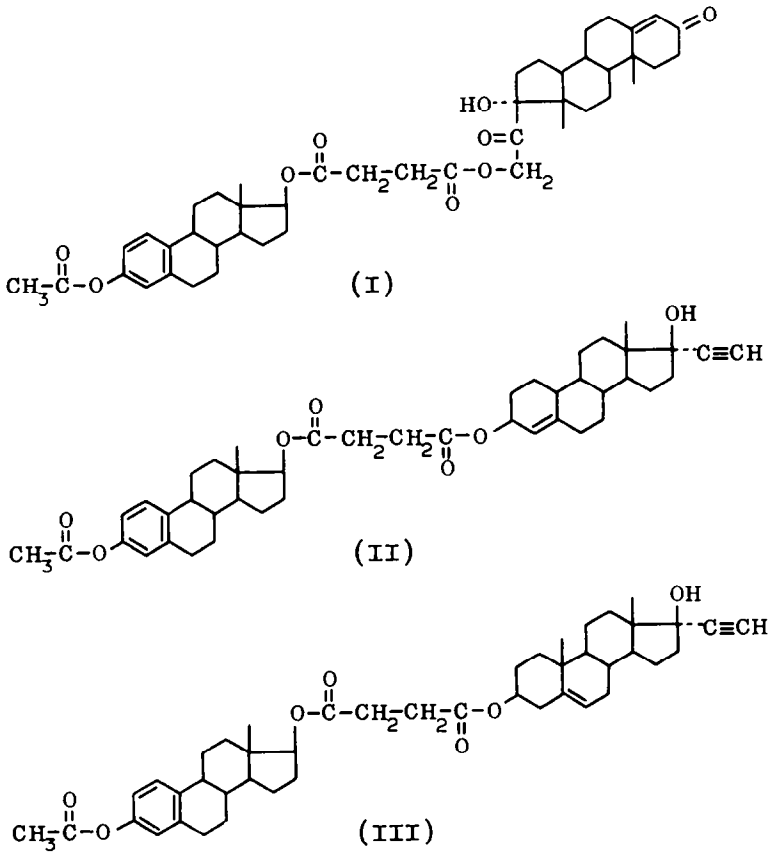
CHEMISTRY

It has previously been reported (Kuhl and Taubert (1)) that oligomeric steroid esters can be prepared by direct esterification of steroid hemidicarboxylic acid esters with steroid alcohols.

This reaction utilizes N,N'-carbonyldiimidazole in accordance with the procedure described by Staab and Mannschreck (2) to transform the steroid hemidicarboxylic acid ester into a reactive intermediate, i. e. the respective carboxylic acid imidazolide. The addition of the steroid alcohol to the reaction mixture results in the formation of dimeric or oligomeric steroid esters.

This method has the particular advantage in that it permits the combination of different types of steroids to give oligomeric compounds. It has thus been possible to link estradiol to cortexolone (Reichstein S), to ethinylandrostenediol, and to ethynodiol. In each case 3-acetoxy-estradiol-17ß-hemisuccinate was used as the carboxylic acid component to combine with the respective steroid alcohols after undergoing the initial reaction with N,N'-carbonyldiimidazole.

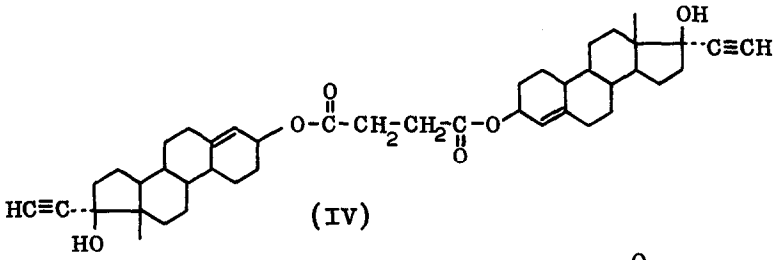
The reaction of 3-acetoxy-estradiol-17ß-hemisuccinate with excess cortexolone gave the dimeric compound (I). In this case, the coupling reaction took place at the easily esterifiable C_{21}-OH-group of cortexolone, while the 17α-OH-group remained unaffected due to steric hindrance.



17α-ethinyl-5-androstene-3ß,17ß-diol and the potent progestogen ethynodiol were coupled in a like manner. The esterification of these steroids with the estradiol derivative occurred only at the easily accessible 3ß-OH-groups. The 17ß-OH-groups remained uninvolved during this reaction.

The hydrolysis of both the estradiol-ethynodiol-derivative (II) and the combination of estradiol and ethinyl-androstenediol (III) by means of KOH/methanol resulted

in their splitting into the parent compounds estradiol, ethynodiol, and ethinylandrostenediol respectively. The synthesis of a long-acting progestogen by the process of dimerization could be demonstrated by means of ethynodiol. The reaction of ethynodiol-3ß-hemisuccinate with N,N'-carbonyldiimidazole and an excess of ethynodiol gave the dimeric ethynodiol derivative (IV), where both steroid molecules are linked at C_3.



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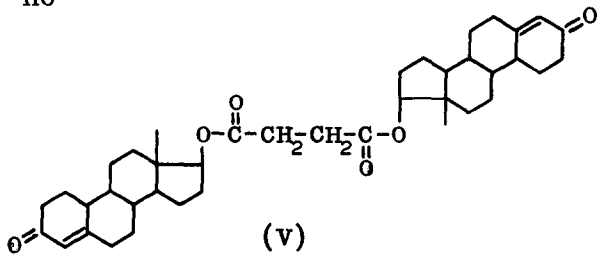

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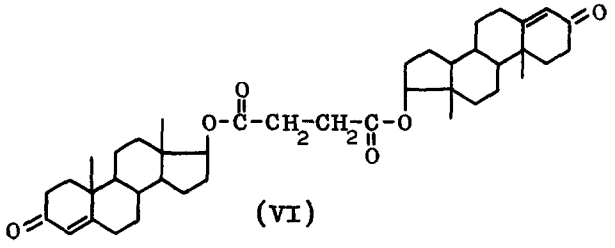
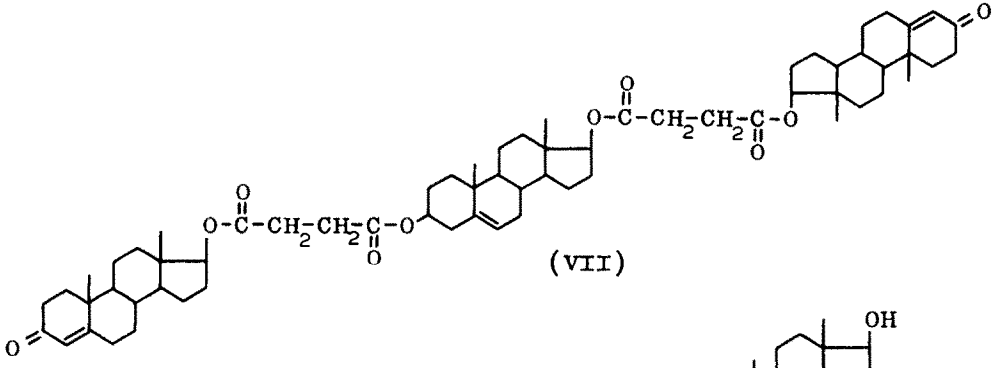


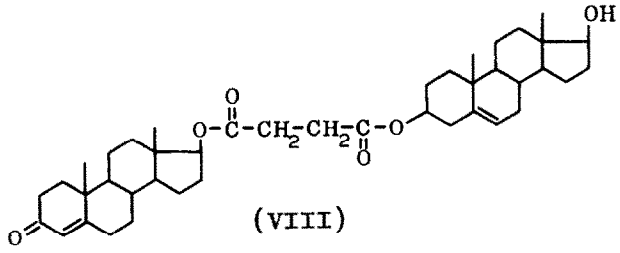
(VI)

The same principle was applied to the synthesis of dimeric nortestosterone (V) and dimeric testosterone (VI).

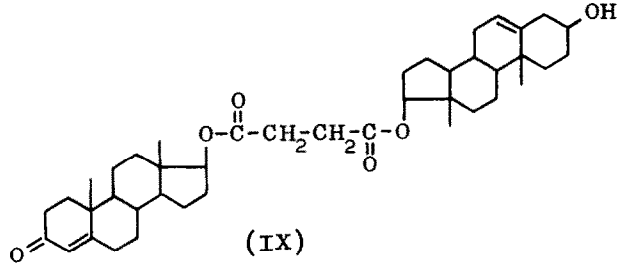
As testosterone contains only one hydroxyl group at C_{17}, only dimeric derivatives can be synthesized. As a consequence, an androgen possessing two free hydroxyl groups would be required to synthesize a trimeric androgen. The preparation of such a compound was achieved by esterifying the hydroxyl groups at position C_3 and C_{17} of androstenediol with excess testosterone hemisuccinate. From the reaction mixture a trimeric (VII) and two dimeric compounds (VIII and IX) could be isolated.



(VII)



(VIII)



(IX)

The yield was 31% for compound (VII) and 29% for compound (VIII) as compared to only 10% for compound (IX). This is interpreted as meaning that the 3ß-OH-group is more easily esterifiable as the 17ß-OH-group whose accessibility is affected by the angular C_{18}-methyl group.

BIOLOGY

The two dimeric compounds (I) and (II) were tested only in respect to their estrogenic activity. It was determined in rats (body-weight 200-250 g) by means of the Allen-Doisy-Test (3). The estradiol-cortexolone-derivative (I) brought about vaginal estrus changes for an average of 19 days, when 6 animals were given a single injection of 20 µg in 0.5 ml arachis oil/benzyl benzoate (6:4). The injection of 40 µg of the estradiol-ethynodiol derivative into 20 rats was followed by estrus for a mean of 51 days.

An antiestrogen-Test (4) was used to determine the period of effectiveness of dimeric ethynodiol (IV). Groups of rats each comprising 6 animals were used. They received at first a single injection of 40 µg of the long-acting dimeric estradiol derivative [17ß-acetoxy-1,3,5(10)-estratrien-3-yl]-[3-acetoxy-1,3,5(10)-estratrien-17ß-yl]-succinate (Kuhl and Taubert (1)) to induce a long-lasting stimulation of the vaginal epithelium. After estrus smears

had been obtained, each rat was given the respective dose of the test compound in 0.5 ml arachis oil/benzyl benzoate (6:4).

Compound	Dose	Duration of Effect
progesterone	10 mg	7 days
ethynodiol diacetate	10 mg	5 days
dimeric ethynodiol	10 mg	30 days
dimeric ethynodiol	20 mg	55 days

The androgenic effectiveness of the dimeric testosterone (VI) was tested in castrated male rats (body-weight 280-300 g). They received a single i.m. injection of 10 mg of dimeric testosterone (DT) or 10 mg of testosterone enanthate (TE) in 0.4 ml arachis oil, and the weight of the prostate gland and the seminal vesicles was determined in groups each comprised of 10 rats at varying time intervals after the injection in accordance with the method previously described (5).

Time after injection	Prostate gland (mg) (DT)	(TE)	Seminal vesicles (mg) (DT)	(TE)
Controls	16 ± 2		33 ± 10	
8 days	231 ± 18	311 ± 60	229 ± 48	707 ± 133
16 days	180 ± 71	392 ± 111	464 ± 156	953 ± 140
32 days	302 ± 82	499 ± 82	299 ± 140	754 ± 231
64 days	166 ± 46	149 ± 64	262 ± 161	224 ± 104
108 days	105 ± 50	67 ± 22	171 ± 85	102 ± 18

After 108 days, the organ weights of the animals treated with dimeric testosterone were found to be higher than that of animals which had been treated with an equal amount of testosterone enanthate.

EXPERIMENTAL

All melting points were determined on a Tottoli melting point apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer 137 spectrophotometer (KBr-method). Nuclear magnetic resonance spectra were recorded on a Varian-HA NMR spectrometer (100 MHz) with tetramethylsilane as internal standard. Elemental analyses were performed by Dr. W. Rozdzinski, University of Stuttgart.

Estradiol-Cortexolone (I)

[3-Acetoxy-1,3,5(10)-estratrien-17β-yl]-[17-hydroxy-3,20-dioxo-4-pregnen-21-yl]-succinate

1.66 g (4 mMol) [3-acetoxy-1,3,5(10)-estratrien-17β-yl]-hemisuccinate and 1.3 g (8 mMol) N,N'-carbonyldiimidazole were dissolved in 60 ml dry tetrahydrofurane and the mixture was left for five hours at room temperature. Then 6.9 g (20 mMol) 17α,21-dihydroxy-4-pregnene-3,20-dione was added. After standing for three days at room temperature the solution was refluxed for two hours. Then the mixture was diluted with 300 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate, and evaporated. The residue was chromatographed on silica gel with chloroform. The compound eluated second was evaporated and recrystallized from acetone/n-hexane, yielding 0.72 g.
m.p. 203-204°, yield 24%.
IR-spectrum: 3390 cm^{-1} (17α-OH), 1770 cm^{-1} (C=O phenol ester), 1750 cm^{-1} (C=O 17β-ester of estradiol and 21-ester of cortexolone), 1730 cm^{-1} (C=O 20-oxo), 1668 cm^{-1} (C=O 3-oxo, α,β-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.70 (3 H of C_{18}-methyl group of cortexolone, adjacent to 17α-OH-group), δ = 0.82 (3 H of C_{18}-methyl group of estradiol, adjacent to 17β-ester), δ = 1.16 (3 H of C_{19}-methyl group of cortexolone, influenced by Δ_4-3-oxo group), δ = 2.23 (3 H of phenol acetate).
$C_{45}H_{58}O_9$ (742.91) calcd.: 72.75 %C; 7.87 %H;
found : 72.85 %C; 7.81 %H.

Estradiol-Ethynodiol (II)

[3-Acetoxy-1,3,5(10)-estratrien-17β-yl]-[17β-hydroxy-17α-ethinyl-4-estren-3β-yl]-succinate

1.25 g (3 mMol) [3-acetoxy-1,3,5(10)-estratrien-17β-yl]-hemisuccinate and 0.49 g (3 mMol) N,N'-carbonyldiimidazole were dissolved in 15 ml dry tetrahydrofurane and the mix-

ture was left overnight at room temperature. Then 3 g (10 mMol) 17α-ethinyl-4-estrene-3β,17β-diol was added. After standing for eight days at room temperature the solution was refluxed for three hours. The mixture was diluted with 100 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with chloroform, and the two compounds eluated first were evaporated together and chromatographed on silica gel with cyclohexane/chloroform /glacial acetic acid (9:0.5:0.5). The fractions with the compound eluated second were evaporated. The residue was washed with water to remove any remaining acetic acid, and recrystallized from acetone/methanol, yielding 0.28 g. m.p. 95-99°, yield 13%.
IR-spectrum: 3500 cm^{-1} (17β-OH), 3280 cm^{-1} (≡CH of 17α-ethinyl group), 1760 cm^{-1} (C=O phenol acetate), 1725 cm^{-1} (C=O succinate).
NMR-spectrum ($CDCl_3$): δ = 0.78 (3 H of C_{18}-methyl group of ethynodiol, adjacent to 17β-OH and 17α-ethinyl group), δ = 0.82 (3 H of C_{18}-methyl group of estradiol, adjacent to 17β-ester), δ = 2.22 (3 H of phenol acetate), δ = 2.46 (H of 17α-ethinyl group).
$C_{44}H_{56}O_7$ (696.89) calcd.: 75.83 %C; 8.09 %H;
found : 75.73 %C; 8.14 %H.

Estradiol-Ethinylandrostenediol (III)

[3-Acetoxy-1,3,5(10)-estratrien-17β-yl]-[17β-hydroxy-17α-ethinyl-5-androsten-3β-yl]-succinate

1.25 g (3 mMol) [3-acetoxy-1,3,5(10)-estratrien-17β-yl]-hemisuccinate and 0.97 g (6 mMol) N,N'-carbonyldiimidazole were dissolved in 20 ml dry tetrahydrofurane and the mixture was left overnight at room temperature. Then 6.29 g (20 mMol) 17α-ethinyl-5-androstene-3β,17β-diol was added. After standing for five days at room temperature the mixture was diluted with 200 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with chloroform. The fractions with the compound eluated second were evaporated, and the residue was recrystallized from cyclohexane, yielding 0.45 g.
m.p. 141-145°, yield 21%.
IR-spectrum: 3500 cm^{-1} (17β-OH), 3250 cm^{-1} (≡CH of 17α-ethinyl group), 1750 - 1725 cm^{-1} (C=O phenol acetate and C=O of succinate).
$C_{45}H_{58}O_7$ (710.91) calcd.: 76.02 %C; 8.22 %H;
found : 76.20 %C; 8.32 %H.

Dimeric Ethynodiol (IV)

Bis-(17β-hydroxy-17α-ethinyl-4-estren-3β-yl)-succinate

2.8 g (7 mMol) (17β-hydroxy-17α-ethinyl-4-estren-3β-yl)-hemisuccinate and 1.62 g (10 mMol) N,N'-carbonyldiimidazole were dissolved in 50 ml dry tetrahydrofurane and the mixture was left overnight at room temperature. Then 6 g (20 mMol) 17α-ethinyl-4-estrene-3β,17β-diol was added. After standing for five days at room temperature the mixture was diluted with 1 l water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with chloroform/acetone (9:1) and recrystallized from cyclohexane, yielding 1.6 g.
m.p. 161-163°, yield 33%.
IR-spectrum: 3500 cm^{-1} (17β-OH), 3300 cm^{-1} (≡CH of 17α-ethinyl group), 1725 cm^{-1} (C=O of succinate).
NMR-spectrum ($CDCl_3$): δ = 0.86 (6 H of C_{18}-methyl groups, adjacent to 17β-OH and 17α-ethinyl), δ = 2.0 (2 H of the 17β-OH groups), δ = 2.51 (2 H of ethinyl groups), δ = 2.58 (4 H of succinate).
$C_{44}H_{58}O_6$ (682.90) calcd.: 77.38 %C; 8.56 %H;
found : 77.32 %C; 8.63 %H.

Dimeric Nortestosterone (V)

Bis-(3-oxo-4-estren-17β-yl)-succinate

2.1 g (5.5 mMol) (3-oxo-4-estren-17β-yl)-hemisuccinate and 2 g (12 mMol) N,N'-carbonyldiimidazole were dissolved in 30 ml dried tetrahydrofurane. When the development of CO_2 had been terminated, 3 g (11 mMol) 17β-hydroxy-4-estrene-3-one was added. After standing overnight at room temperature the solution was refluxed for six hours. Then the mixture was diluted with 300 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with cyclohexane/ethyl acetate (6:4) and the compound (V) recrystallized from acetone/n-hexane, yielding 0.41 g.
m.p. 190-191°, yield 12%.
IR-spectrum: 1730 cm^{-1} (C=O of succinate), 1670 cm^{-1} (C=O 3-oxo, α,β-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.76 (6 H of C_{18}-methyl groups, adjacent to 17β-ester), δ = 2.50 (4 H of succinate), δ = 5.72 (2 olefinic protons at C_4).
$C_{40}H_{54}O_6$ (630.83) calcd.: 76.15 %C; 8.63 %H;
found : 75.98 %C; 8.55 %H.

Dimeric Testosterone (VI)

Bis-(3-oxo-4-androsten-17β-yl)-succinate

0.78 g (2 mMol) (3-oxo-4-androsten-17β-yl)-hemisuccinate and 0.81 g (5 mMol) N,N'-carbonyldiimidazole were dissolved in 20 ml dry tetrahydrofurane. When the development

of CO_2 had been terminated, 2.88 g (10 mMol) 17ß-hydroxy-4-androstene-3-one was added. After standing overnight at room temperature the solution was refluxed for three hours. Then the mixture was diluted with 200 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with ethyl acetate/chloroform (1:1) and the compound (VI) recrystallized from acetone, yielding 0.53 g.
m.p. 207-209°, yield 40%.
IR-spectrum: 1730 cm^{-1} (C=O of succinate), 1670 cm^{-1} (C=O 3-oxo, α,ß-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.72 (6 H of C_{18}-methyl groups, adjacent to 17ß-ester), δ = 1.08 (6 H of C_{19}-methyl groups, influenced by Δ_4-3-oxo group), δ= 2.5 (4 H of succinate), δ = 5.6 (2 olefinic protons at C_4).
$C_{42}H_{58}O_6$ (658.88) calcd.: 76.56 %C; 8.87 %H;
found : 76.40 %C; 8.80 %H.

Trimeric Androgen

3ß,17ß-Bis-(3-oxo-4-androsten-17ß-yl-oxycarbonyl)-propionyloxy-5-androstene (VII)

(3-Oxo-4-androsten-17ß-yl)-(17ß-hydroxy-5-androsten-3ß-yl)-succinate (VIII)

(3-Oxo-4-androsten-17ß-yl)-(3ß-hydroxy-5-androsten-17ß-yl)-succinate (IX)

7.8 g (20 mMol) (3-oxo-4-androsten-17ß-yl)-hemisuccinate and 3.24 g (20 mMol) N,N'-carbonyldiimidazole were dissolved in 35 ml dry tetrahydrofurane. When the development of CO_2 had been terminated, 1.45 g (5 mMol) 5-androstene-3ß,17ß-diol was added. After standing for three days at room temperature the solution was refluxed for two hours. Then the mixture was diluted with 500 ml water and extracted with chloroform. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel with chloroform/acetone (9:1). Three substances were eluated in the following sequence: (VII), (VIII) and (IX). The fractions were evaporated and recrystallized.
(VII): recrystallized from acetone/methanol,
m.p. 156-159°, yield 1.58 g (31%).
IR-spectrum: 1730 cm^{-1} (C=O of succinates), 1680 cm^{-1} (C=O 3-oxo, α,ß-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.91 (3 H of C_{18}-methyl group of androstenediol, adjacent to 17ß-ester), δ = 0.94 (6 H of C_{18}-methyl group of testosterone, adjacent to 17ß-ester), δ = 1.13 (3 H of C_{19}-methyl group of androstenediol, adjacent to Δ_5), δ = 1.3 (6 H of C_{19}-methyl group of testosterone, adjacent to Δ_4), δ = 2.7 (8 H of succinate).
$C_{65}H_{90}O_{10}$ (1031.37) calcd.: 75.69 %C; 8.80 %H;
found : 75.42 %C; 8.73 %H.

(VIII): recrystallized from cyclohexane/acetone/benzene, m.p. 172-174°, yield 0.97 g (29%).
IR-spectrum: 3500 cm^{-1} (17β-OH), 1730 cm^{-1} (C=O of succinate), 1670 cm^{-1} (C=O 3-oxo, α,β-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.84 (3 H of C_{18}-methyl group of androstenediol, adjacent to 17β-OH), δ = 0.92 (3 H of C_{18}-methyl group of testosterone, adjacent to 17β-ester), δ = 1.13 (3 H of C_{19}-methyl group of androstenediol, adjacent to Δ_5), δ = 1.28 (3 H of C_{19}-methyl group of testosterone, adjacent to Δ_4), δ = 1.62 (1 H of 17β-OH-group), δ = 2.68 (4 H of succinate).
$C_{42}H_{60}O_6$ (660.90) calcd.: 76.32 %C; 9.15 %H;
found : 76.14 %C; 9.24 %H.

(IX): recrystallized from acetone/cyclohexane, m.p. 180-185°, yield 0.33 g (10%).
IR-spectrum: 3430 cm^{-1} (3β-OH), 1730 cm^{-1} (C=O of succinate), 1670 cm^{-1} (C=O 3-oxo, α,β-unsaturated).
NMR-spectrum ($CDCl_3$): δ = 0.92 (3 H of C_{18}-methyl group of androstenediol, adjacent to 17β-ester), δ = 0.95 (3 H of C_{18}-methyl group of testosterone, adjacent to 17β-ester), δ = 1.13 (3 H of C_{19}-methyl group of androstenediol, adjacent to Δ_5), δ = 1.31 (3 H of C_{19}-methyl group of testosterone, adjacent to Δ_4), δ = 1.72 (1 H of 3β-OH-group), δ = 2.72 (4 H of succinate).
$C_{42}H_{60}O_6$ (660.90) calcd.: 76.32 %C; 9.15 %H;
found : 76.26 %C; 8.92 %H.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Dr. Krämer, Dr. Orth (Div. Chem. Research) and Dr. Pohl (Dept. Phys. Chem.) of E. Merck, Darmstadt, Germany, for their support of this study by supplying the data of NMR-spectra.
The support of parts of this study by a grant of the WHO is also gratefully acknowledged.

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