# ADDITION OF AZOIMIDE TO UNSATURATED KETONES IN THE STEROID SERIES. SYNTHESIS OF *N*-(17β-HYDROXY-3-OXO-5α-ANDROSTAN-15β-YL)-SUCCINAMOIC ACID AND ITS EVALUATION AS HAPTEN FOR DIHYDROTESTOSTERONE IMMUNOANALYSIS*

Ivan CERNY[a1], Vladimir POUZAR[a2], Oldrich LAPCIK[b] and Richard HAMPL[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1]cerny@uochb.cas.cz, [2]pouzar@uochb.cas.cz*

[b] *Institute of Endocrinology, 116 94 Prague 1, Czech Republic; e-mail: rhampl@endo.lib.cas.cz*

Received September 24, 1997
Accepted November 12, 1997

Addition of azoimide to 17-oxoandrosta-5,15-dien-3β-yl acetate or 17-oxo-5α-androst-15-en-3β-yl acetate gave in both cases corresponding 15β-azido derivatives. 15β-Azido-17-oxo-5α-androstan-3β-yl acetate was selectively reduced to 17β-hydroxy derivative and protected as methoxymethyl ether. Subsequent reduction of azide group and condensation with methyl hydrogen succinate gave a protected succinylamino derivative. Deacetylation and oxidation then led to dihydrotestosterone (DHT) series. Successive removal of protecting groups gave *N*-(17β-hydroxy-3-oxo-5α-androstan-15β-yl)succinamoic acid, an experimental hapten for DHT. Its conjugate with bovine serum albumin was used for immunization of rabbits and corresponding antisera were evaluated in RIA using [$^3$H]-DHT as a tracer. The results are comparable with standard kits based on antisera generated using DHT derivatives with connecting bridge in position 7.

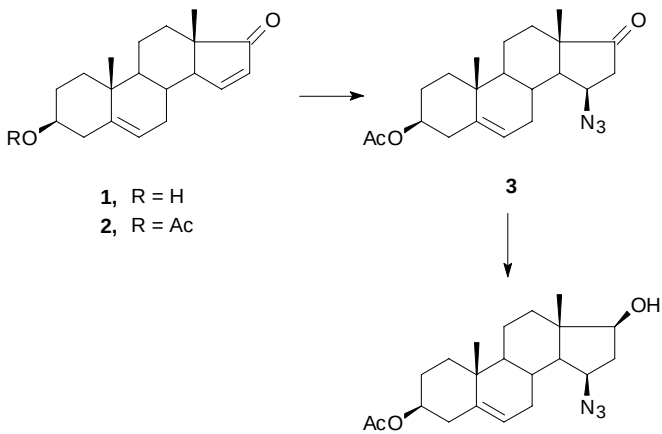
**Key words:** Steroids; Azoimide; DHT; Haptens.

Continuing our work on 15-substituted derivatives of the androstane series, we have focused on 15-amino derivatives. For the introduction of the nitrogen substituent into steroid molecule, we adopted the reaction of azoimide with conjugated enones[2]. One of the potential uses of such derivatives is their transformation into haptens with the amidic connecting bridge[3].

The preliminary experiments were done on 3β-hydroxyandrosta-5,15-dien-17-one[4] (**1**) with generation of azoimide *in situ* from sodium azide and acetic acid. The starting compound was dissolved in acetic acid, saturated aqueous sodium azide was added, and the reaction mixture was stirred at room temperature for four hours. After the work-up, we obtained a mixture, from which after acetylation and column chromatography 15β-

* Part CCCXCII in the series On Steroids; Part CCCXCI see ref.[1].

azido-17-oxoandrost-5-en-3β-yl acetate (**3**) was separated as the main product. Under these conditions the reaction was not complete, probably due to heterogeneity of the reaction mixture. Moreover, the TLC monitoring was difficult because the product and starting compound had similar mobility. After several additional experiments, we found that both these drawbacks can be eliminated by using acetate **2** as a starting compound and by addition of tetrahydrofuran into the reaction mixture (see Experimental). Such a modified reaction was used for further experiments. The subsequent reduction step was also modelled in the 5-ene series: sodium borohydride in a methanol–ethyl acetate mixture smoothly reduced ketone **3** to 17β-hydroxy derivative **4** (Scheme 1).




**1**, R = H
**2**, R = Ac

**3**

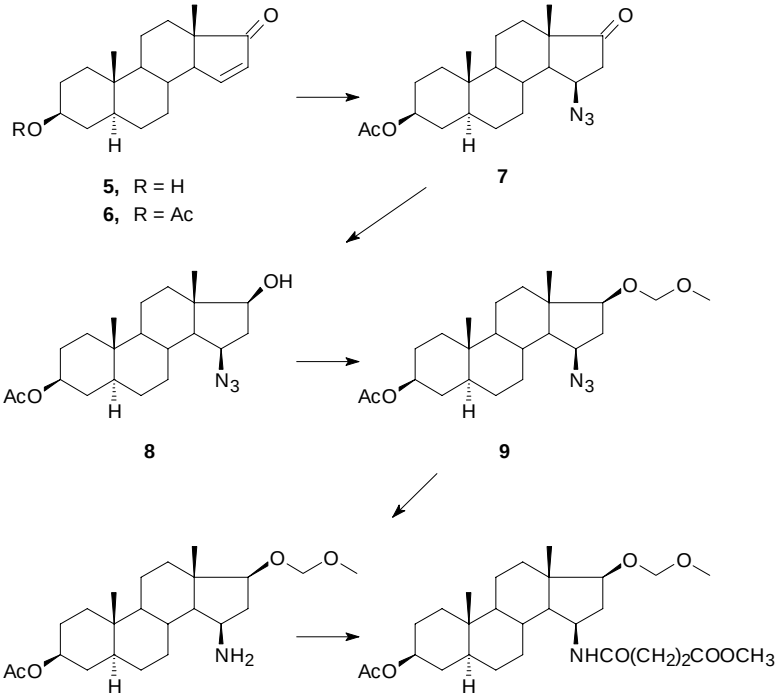
**4**

SCHEME 1

In the 5α-androstane series, we reacted 3β-hydroxy-5α-androstan-15-en-17-one[5] (**5**) with acetic anhydride in pyridine to give acetate **6**. This compound was, without purification, subjected to the azoimide addition and after column chromatography and crystallization from ethanol, we obtained 15β-azido-17-oxo-5α-androstan-3β-yl acetate (**7**) in 65% yield. The borohydride reduction of ketone **7** gave 17β-hydroxy derivative **8** which was subsequently protected by reaction with chloromethyl methyl ether yielding methoxymethyl ether **9**.

For the preparation of amine **10**, we tried to use hydrogenation of **9** over palladium on activated carbon in acetic acid, but deactivation of the catalyst during reaction made this method unusable. Of the other methods we succeeded in reducing **9** with sodium borohydride in the presence of nickel(II) chloride[6]: we obtained 15β-amino-17β-(methoxymethoxy)-5α-androstan-3β-yl acetate (**10**) as a semisolid mass (Scheme 2).

This product was condensed with methyl hydrogen succinate in the presence of *N*,*N*′-dicyclohexylcarbodiimide and afforded amide **11** which was, without further purification, deacetylated with sodium methoxide in methanol to methyl *N*-[3β-hydroxy-



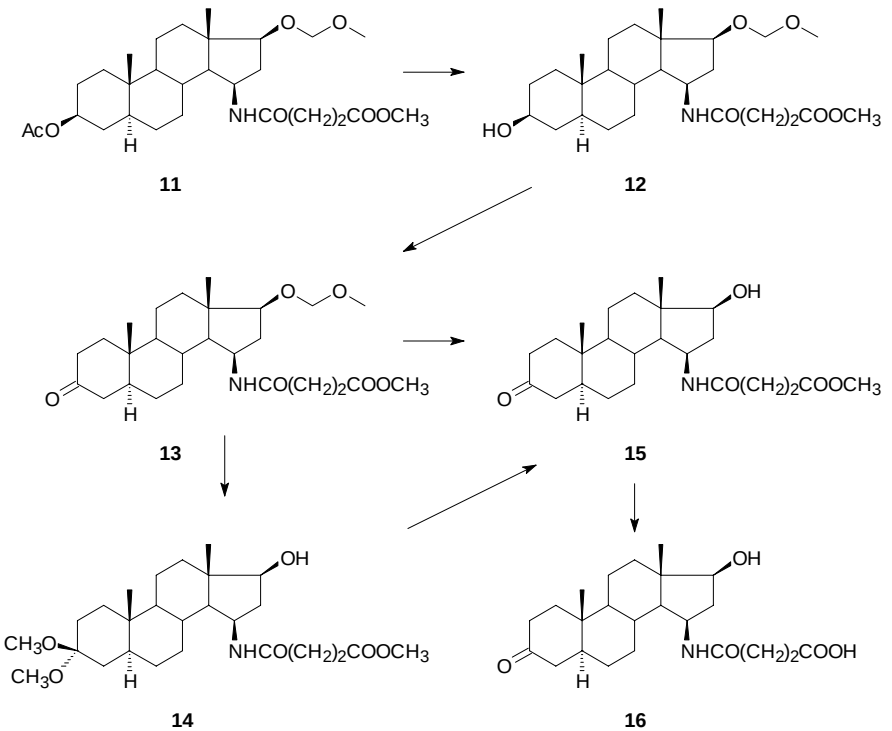

SCHEME 2

17β-(methoxymethoxy)-5α-androstan-15β-yl]succinamate (**12**). Subsequent oxidation with the Jones reagent completed transformation into the dihydrotestosterone (DHT) series giving methyl *N*-[17β-(methoxymethoxy)-3-oxo-5α-androstan-15β-yl]succinamate (**13**) (Scheme 3).

The removal of protecting groups was performed in two steps. The 4-toluenesulfonic acid monohydrate in a benzene–methanol mixture split off the methoxymethyl group. As a side product, the corresponding dimethyl ketal **14** was observed in $^1$H NMR spectrum of the crude product from this reaction (3.19 s and 3.14 s: $CH_3O$–C(3)–$OCH_3$; 0.95 s and 0.85 s: H-18 and H-19). This step was therefore completed by treatment with 4-toluenesulfonic acid monohydrate in acetone to give **15**. In the second step aqueous-methanolic potassium hydroxide saponified the ester. The *N*-(17β-hydroxy-3-oxo-5α-androstan-15β-yl)succinamoic acid (**16**) obtained was preliminary tested as a hapten for DHT immunoassays.

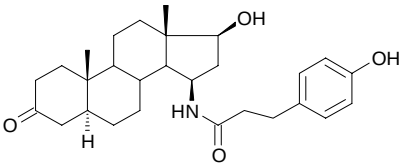
The conjugate with bovine serum albumin (BSA) was prepared by the standard method[7] and the amount of hapten **16** bound was assayed by the titration of free amino groups with 2,4,6-trinitrobenzenesulfonic acid[8] to yield 14 units per molecule of BSA. Rabbits were immunized by 250 μg of the conjugate four times in 4–6 weeks periods.

The antisera obtained (Serum No. 21 and 22) were found to bind [$^3$H]-DHT and displayed a good sensitivity in DHT radioimmunoassay using [$^3$H]-DHT as indicator (Table I). However, the cross-reactivity with testosterone is too high for direct use in DHT assays. Nevertheless, it is comparable with the routinely used systems based on DHT haptens with bridge in position 7, where the analysis must be combined with oxidative treatment removing interfering testosterone before the assay.

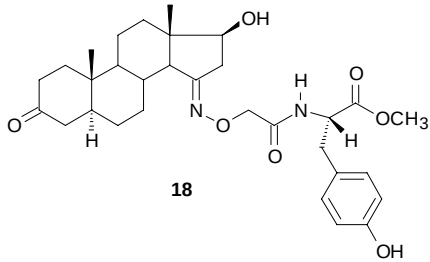



SCHEME 3

Additionally, we tested the antisera in assays using $^{125}$I labelled indicators. We used compound **17**, corresponding to condensation of amine **10** with activated 3-(4-hydroxyphenyl)propionate (Bolton–Hunter reagent[9]) for homologous assay and L-tyrosine methyl ester conjugate of (15*E*)-17β-hydroxy-5α-androstane-3,15-dione 15-(*O*-carboxymethyl)oxime[1] (**18**) for heterologous assay. Both compounds were radioiodinated by the same method[10] and radioligands were purified by TLC, giving ligand A and ligand B, respectively. For the hapten **17**, the radioligand (ligand A) could not be displaced by nonradioactive DHT, probably due to a strong bridge effect, so that this system could not be used in the DHT assay (Table I). The ligand B, derived from 15-CMO DHT **18**, was useful as a tracer in DHT RIA although the results were not better than those in assays using [$^3$H]-DHT (Table I).




**17**




**18**

In summary, we developed a method for preparation of 15β-amino derivatives in the androstane series and made use of it in preparation of a model hapten for DHT with amidic bridge. Previous immunoassays provided active antisera generated with this hapten, but, compared with classical methods, a mere change of connecting position could not avoid the interference of 3-oxo-4-ene steroids.

## EXPERIMENTAL

Melting points were determined on a Boetius micromelting point apparatus (Jena, Germany). Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter; $[\alpha]_D$ values are given in ° [$10^{-1}$ deg cm$^2$ g$^{-1}$]. Infrared spectra (wavenumbers in cm$^{-1}$) were recorded on a Bruker IFS 88 spectrometer in chloroform. $^1$H NMR spectra were taken on a Varian UNITY-200 (200 MHz) or a Varian UNITY-500 (500 MHz) spectrometer at 23 °C in deuteriochloroform with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and multiplet widths (*W*) in Hz. The purity of the products and reaction course were checked by thin-layer chromatography (TLC) performed on silica gel G (ICN Biochemicals) followed by spraying with concentrated sulfuric acid and heating. Column chromatography was performed on silica gel (60–120 μm, Service laboratory of the Institute). Prior to evaporation on a rotary evaporator *in vacuo* (bath temperature 50 °C), solutions in organic solvents were dried over magnesium sulfate. Analytical samples were dried over phosphorus pentoxide at 40 °C/26 Pa for 12 h.

15β-Azido-17-oxoandrost-5-en-3β-yl Acetate (**3**)

Ketone **1** (1.0 g, 3.49 mmol) was acetylated with acetic anhydride (2 ml, 21.2 mmol) in pyridine (20 ml) overnight. The mixture was poured on ice and extracted with ethyl acetate. The organic layer was washed with 10% HCl, water, saturated aqueous $KHCO_3$ and water, dried, and the solvents were evaporated. Resulting acetate **2**, without purification, was dissolved in tetrahydrofuran (10 ml) and acetic acid (10 ml). To the stirred solution was added sodium azide (2 g, 30.8 mmol) in water (5 ml). After 6 h, the mixture was poured on cold saturated aqueous $KHCO_3$ and extracted with ethyl acetate (100 ml). The organic phase was washed with saturated aqueous $KHCO_3$, dried, and solvents were evaporated. The residue was crystallized from hot ethanol to give 930 mg (72%) of azide **3**, m.p. 162–164 °C, $[\alpha]_D$ –62° (*c* 1.2, chloroform). IR spectrum (chloroform): 2 116, 2 088 (azide); 1 734 (C=O, acetate and ketone); 1 670 (C=C); 1 367 ($CH_3$, acetate); 1 264, 1 025 (C–O, acetate); 1 076 (C–O). $^1$H NMR spectrum: 5.42 m, 1 H, *W* = 10 (H-6); 4.61 m, 1 H, *W* = 32 (H-3α); 4.33 ddd, 1 H, *J* ≈ 7.0, 6.0, 2.0 (H-15α); 2.72 dd, 1 H, *J* ≈ 20.0, 2.0 (H-16β); 2.55 dd, 1 H, *J* ≈ 20.0, 6.5 (H-16α); 2.04 s, 3 H ($CH_3COO$); 1.10 s, 3 H (3 × H-18); 1.09 s, 3 H (3 × H-19). For $C_{21}H_{29}N_3O_3$ (371.5) calculated: 67.90% C, 7.87% H, 11.31% N; found: 68.09% C, 8.00% H, 11.57% N.

15β-Azido-17-hydroxyandrost-5-en-3β-yl Acetate (**4**)

To a stirred solution of **3** (1.5 g, 4.0 mmol) in a mixture of ethyl acetate (15 ml) and methanol (30 ml), sodium borohydride (180 mg, 4.8 mmol) was gradually added at 10 °C during 5 min. After additional 5 min of stirring at the same temperature, the excess hydride was destroyed by acetic acid (0.3 ml) and water (0.3 ml). The solution was concentrated under reduced pressure, diluted with ethyl acetate, and washed successively with saturated aqueous NaCl, 10% HCl, water, saturated aqueous $KHCO_3$, and water. After drying and evaporation, the residue was crystallized from ethanol–petroleum ether giving 1.08 g (72%) of azide **4**, m.p. 121–122 °C, $[\alpha]_D$ –112° (*c* 0.6, chloroform). IR spectrum (chloroform): 3 610, 3 484 (O–H); 2 110, 2 085 (azide); 1 723 (C=O, acetate); 1 671 (C=C); 1 366 ($CH_3$, acetate); 1 255, 1 047, 1 029 (C–O). $^1$H NMR spectrum: 5.40 m, *W* = 12 (H-6); 4.61 m, 1 H, *W* = 32 (H-3α); 3.96 ddd, 1 H, *J* ≈ 8.5, 6.5, 3.0 (H-15α); 3.63 t, 1 H, *J* ≈ 8.5 (H-17α); 2.62 dt, 1 H, *J* ≈ 15.0, 2 × 8.5 (H-16α); 2.04 s, 3 H ($CH_3COO$); 1.71 ddd, 1 H, *J* ≈ 15.0, 8.5, 3.0 (H-16β); 1.07 s, 3 H (3 × H-19); 0.92 s, 3 H (3 × H-18). For $C_{21}H_{31}N_3O_3$ (373.5) calculated: 67.53% C, 8.37% H, 11.25% N; found: 67.24% C, 8.48% H, 11.13% N.

15β-Azido-17-oxo-5α-androstan-3β-yl Acetate (**7**)

Ketone **5** (1.0 g, 3.47 mmol) was processed as in the preparation of **3** to give 1.1 g (86%) of crude azide **7**. The chromatography on a silica gel column (150 ml) in a benzene–petroleum ether–acetone (50 : 50 : 1) mixture and crystallization from ethanol gave 730 mg (57%) of **7**, m.p. 95–98 °C, $[\alpha]_D$ –18° (*c* 1.1, chloroform). IR spectrum (chloroform): 2 115, 2 088 (azide); 1 736 (C=O, ketone); 1 728 (C=O, acetate); 1 367 ($CH_3$, acetate); 1 255, 1 025 (C–O, acetate). $^1$H NMR spectrum: 4.69 m, 1 H,

TABLE I
Properties of the antisera induced by haptene **16** – bovine serum albumin conjugate in radioimmunoassays

| Technique | Serum No. | |
|---|---|---|
| | 21 | 22 |
| RIA with [$^3$H]-DHT: | | |
| dilution | 1 : 6 4000 | 1 : 3 200 |
| 50% intercept | 30.7 pg/tube | 19.4 pg/tube |
| sensitivity[a] | <1 pg/tube | |
| cross-reactivity[b] | 36.1% | 27.8% |
| RIA with ligand A: | | |
| dilution | 1 : 320 000 | 1 : 160 000 |
| 50% intercept | 808 pg/tube | 377 pg/tube |
| RIA with ligand B: | | |
| dilution | 1 : 2 000 | 1 : 3 000 |
| 50% intercept | 156.8 pg/tube | 59.4 pg/tube |
| cross-reactivity[b] | 39.9% | 27.8% |

[a] Expressed as an amount of the analyte distinguishable with 95% probability from a zero sample. [b] Cross-reactivity against testosterone.

*W* = 32 (H-3α); 4.33 ddd, 1 H, *J* = 5.2, 1.7, 6.5 (H-15α); 2.68 dd, 1 H, *J* = 19.8, 1.7 (H-16β); 2.54 dd, 1 H, *J* = 19.8, 6.5 (H-16α), 2.03 s, 3 H ($CH_3COO$); 1.08 s, 3 H (3 × H-18); 0.89 s, 3 H (3 × H-19). For $C_{21}H_{31}N_3O_3$ (373.5) calculated: 67.53% C, 8.37% H, 11.25% N; found: 67.81% C, 8.48% H, 11.39% N.

15β-Azido-17β-hydroxy-5α-androstan-3β-yl Acetate (**8**)

Ketone **7** (900 mg, 2.41 mmol) in a mixture of ethyl acetate (5 ml) and methanol (10 ml) was processed as in the preparation of **4** using sodium borohydride (120 mg, 3.2 mmol) to give 890 mg (99%) of **8** as foam, $[\alpha]_D$ –70° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 610 (O–H); 2 110, 2 087 (azide); 1 723 (C=O, acetate); 1 367 ($CH_3$, acetate); 1 256, 1 026 (C–O). $^1$H NMR spectrum: 4.67 m, 1 H, *W* = 32 (H-3α); 3.96 ddd, 1 H, *J* = 8.5, 6.4, 3.1 (H-15α); 3.59 t, 1 H, *J* = 8.5 (H-17α); 2.59 dt, 1 H, *J* = 14.5, 2 × 8.5 (H-16α); 2.01 s, 3 H ($CH_3COO$); 0.89 s, 3 H (3 × H-18); 0.86 s, 3 H (3 × H-19). For $C_{21}H_{33}N_3O_3$ (375.5) calculated: 67.17% C, 8.86% H, 11.19% N; found: 67.31% C, 8.93% H, 11.21% N.

15β-Azido-17β-(methoxymethoxy)-5α-androstan-3β-yl Acetate (**9**)

Hydroxy derivative **8** (850 mg, 2.26 mmol) was dissolved in dichloromethane (10 ml) and *N*,*N*-diisopropylethylamine (1.5 ml, 10.7 mmol). To a stirred solution chloromethyl methyl ether (0.4 ml, 5.3 mmol) was added dropwise and the stirring continued at room temperature for 4 h. Then the reaction mixture was poured on ice cold saturated aqueous NaCl and extracted with ether (50 ml). The ethereal layer was washed twice with 10% HCl, saturated aqueous $KHCO_3$ and saturated aqueous NaCl. After drying, the solution was filtered through a short column of aluminum oxide and concentrated giving 1 g (105%) of oily **9**, which successively solidified. Recrystallization from ethanol yielded 800 mg (84%) of **9**, m.p. 84–85 °C, $[\alpha]_D$ –74° (*c* 1.0, chloroform). IR spectrum (chloroform): 2 110, 2 089 sh (azide); 1 724 (C=O, acetate); 1 255, 1 027 (C–O, acetate); 1 150, 1 103, 1 045 (C–O, $CH_3OCH_2O$); 913 ($CH_2$, $CH_3OCH_2O$). $^1$H NMR spectrum: 4.68 m, 1 H, *W* = 35 (H-3α); 4.63 s, 2 H ($OCH_2O$); 3.97 ddd, 1 H, *J* = 8.5, 6.4, 2.8 (H-15α); 3.46 t, 1 H, *J* = 8.5 (H-17α); 3.36 s, 3 H (C**H**$_3OCH_2$); 2.58 dt, 1 H, *J* = 14.7, 2 × 8.5 (H-16α); 2.02 s, 3 H ($CH_3COO$); 0.93 s, 3 H (3 × H-18); 0.86 s, 3 H (3 × H-19). For $C_{23}H_{37}N_3O_4$ (419.6) calculated: 65.84% C, 8.89% H, 10.02% N; found: 66.06% C, 8.91% H, 10.12% N.

Methyl *N*-[3β-Hydroxy-17β-(methoxymethoxy)-5α-androstan-15β-yl]succinamate (**12**)

To a stirred solution of azide **9** (600 mg, 1.43 mmol) in a mixture of ethanol (20 ml) and dioxane (20 ml), sodium borohydride (240 mg, 6.34 mmol) and then dropwise 0.1 M ethanolic solution of nickel chloride hexahydrate (1.4 ml, 0.14 mmol) were added. During the addition of the nickel solution, a black precipitate was formed. After 1 h stirring, acetic acid (*ca* 2 ml) and water (*ca* 1 ml) were added to dissolve most of the catalyst. The mixture was concentrated to a small volume, poured into saturated aqueous $KHCO_3$ (100 ml), and extracted with ethyl acetate (*ca* 70 ml). After drying, the solvents were evaporated and the residue coevaporated with toluene (three times) giving 600 mg (106%) of crude 15β-amino-17β-(methoxymethoxy)-5α-androstan-3β-yl acetate (**10**) as a semisolid paste homogeneous on TLC in a chloroform–propan-2-ol–25% aqueous ammonia (100 : 10 : 0.5) mixture. Amine **10** was dissolved in benzene (10 ml), methyl hydrogen succinate (450 mg, 3.41 mmol) was added and then, after dissolution, 1 M *N*,*N*′-dicyclohexylcarbodiimide in benzene (2 ml, 2 mmol), all under stirring. The stirring continued 1 h and the completion of reaction was checked by TLC in the above mentioned mixture. Two drops of water were added and solvents were evaporated. The solid residue was extracted with ethyl acetate (3 × 15 ml) and the solution was washed with saturated

aqueous $KHCO_3$, saturated aqueous NaCl, dried and evaporated to give the foamy residue containing methyl *N*-[3β-acetoxy-17β-(methoxymethoxy)-5α-androstan-15β-yl]succinamate (**11**). $^1$H NMR spectrum: 6.03 bd, 1 H, *J* = 8.6 (NH); 4.69 m, 1 H, *W* = 35 (H-3α); 4.60 s, 2 H ($OCH_2O$); 4.26 ddt, 1 H, *J* = 7.6, 3.7, 2 × 8.6 (H-15α); 3.69 s, 3 H ($COOCH_3$); 3.48 t, 1 H, J = 8.6 (H-17α); 3.33 s, 3 H ($CH_3OCH_2$); 2.40–2.80 bm, 5 H ($CH_2CH_2$ succinate + H-16α); 2.02 s, 3 H ($CH_3COO$); 0.97 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19).

Acetate **11** was dissolved in absolute methanol (50 ml), methanolic sodium methoxide (1 ml, from *ca* 100 mg Na in 5 ml of methanol) was added, and the mixture was left aside at room temperature for 24 h. Solid $CO_2$ (about 0.7 g) was added and most of the methanol was evaporated. The mixture was partitioned between chloroform (30 ml) and saturated aqueous NaCl, the organic layer was washed with saturated aqueous NaCl, dried, and solvents were evaporated. Chromatography on the column of silica gel (50 g) in a chloroform–methanol (100 : 1) mixture gave 460 mg (69%) of oily succinamate **12**. IR spectrum (chloroform): 3 610 (O–H); 3 454 (N–H); 1 731 (C=O, ester); 1 663, 1 513 (CONH); 1 440 ($CH_3$, ester); 1 154, 1 103, 1 044 (C–O, $CH_3OCH_2O$). $^1$H NMR spectrum: 6.05 bd, 1 H, *J* = 8.6 (NH); 4.60 s, 2 H ($OCH_2O$); 4.25 ddt, 1 H, *J* = 7.6, 3.7, 2 × 8.6 (H-15α); 3.70 s, 3 H ($CH_3OCO$); 3.60 m, 1 H, *W* = 35 (H-3α); 3.48 t, 1 H, *J* = 8.6 (H-17α); 3.34 s, 3 H ($CH_3OCH_2$); 2.40–2.80 bm, 5 H ($CH_2CH_2$ succinate + H-16α); 0.98 s, 3 H (3 × H-18); 0.87 s, 3 H (3 × H-19). For $C_{26}H_{43}NO_6$ (465.5) calculated: 67.07% C, 9.31% H, 3.01% N; found: 67.26% C, 9.03% H, 3.09% N.

Methyl *N*-[17β-(Methoxymethoxy)-3-oxo-5α-androstan-15β-yl]succinamate (**13**)

A solution of hydroxy derivative **12** (210 mg, 0.45 mmol) in acetone (5 ml) was treated with excess Jones reagent. After 5 min at room temperature, the excess reagent was destroyed with methanol (0.2 ml) and the mixture was poured into saturated aqueous $KHCO_3$ and extracted with ethyl acetate. The organic layer was washed twice with saturated aqueous $KHCO_3$ and water, dried, and evaporated. Column chromatography on silica gel (25 g) in a chloroform–methanol (100 : 1) mixture gave 185 mg (88%) of foamy **13**. IR spectrum (chloroform): 3 454, 3 409 (N–H); 1 731 (C=O, ester); 1 707 (C=O, ketone); 1 665, 1 513 (CONH); 1 440 ($CH_3$, ester); 1 153, 1 103, 1 045 (C–O, $CH_3OCH_2O$). $^1$H NMR spectrum: 6.04 bd, 1 H, *J* = 8.6 (NH); 4.61 s, 2 H ($OCH_2O$); 4.28 ddt, 1 H, *J* = 7.6, 3.7, 2 × 8.6 (H-15α); 3.70 s, 3 H ($COOCH_3$); 3.49 t, 1 H, *J* = 8.6 (H-17α); 3.34 s, 3 H ($CH_3OCH_2$); 2.40–2.80 bm, 5 H ($CH_2CH_2$ succinate + H-16α); 1.07 s, 3 H (3 × H-19); 1.00 s, 3 H (3 × H-18). For $C_{26}H_{41}NO_6$ (463.6) calculated: 67.36% C, 8.91% H, 3.02% N; found: 67.48% C, 9.03% H, 3.10% N.

Methyl *N*-(17β-Hydroxy-3-oxo-5α-androstan-15β-yl)succinamate (**15**)

To a solution of amide **13** (165 mg, 0.36 mmol) in a mixture of benzene (5 ml) and methanol (5 ml), 4-toluenesulfonic acid monohydrate (80 mg) was added. The mixture was stirred at 40 °C for 12 h then evaporated to about half of the volume and poured into saturated aqueous $KHCO_3$. The product was extracted with ethyl acetate (30 ml) in three portions. The organic layer was washed with saturated aqueous NaCl and water, dried and solvents were evaporated. The foamy product (160 mg) was dissolved in acetone (5 ml) and a solution of 4-toluenesulfonic acid monohydrate (5 mg) in water (0.5 ml) was added. After 2 h stirring at room temperature, the mixture was poured into saturated aqueous $KHCO_3$ and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried and solvents were evaporated. Crystallization from acetone gave 95 mg (64%) of **15**, m.p. 188–189 °C, $[\alpha]_D$ –3° (*c* 0.9, chloroform). IR spectrum (chloroform): 3 615 (O–H); 3 453, 3 412 (N–H); 1 729 (C=O, ester); 1 707 (C=O, ketone); 1 665, 1 513 (CONH); 1 468, 1 439 ($CH_3$, ester); 1 052 (C–O). $^1$H NMR spectrum: 6.04 bd, 1 H, *J* = 8.7 (NH); 4.28 ddt, 1 H, *J* = 7.6, 3.7, 2 × 8.7 (H-15α); 3.70 s, 3 H ($COOCH_3$); 3.63 t, 1 H, *J* = 8.7 (H-17α); 2.40–2.80 bm, 5 H ($CH_2CH_2$ succi-

nate + H-16α); 1.07 s, 3 H (3 × H-19); 0.98 s, 3 H (3 × H-18). For $C_{24}H_{37}NO_5$ (419.6) calculated: 68.71% C, 8.89% H, 3.34% N; found: 68.89% C, 9.01% H, 3.42% N.

*N*-(17β-Hydroxy-3-oxo-5α-androstan-15β-yl)succinamoic Acid (**16**)

Ester **15** (89 mg, 0.21 mmol) was dissolved in methanol (5 ml), 2 M aqueous sodium hydroxide (2 ml) was added, and the mixture was stirred at room temperature for 1 h. Reaction mixture was acidified with ice-cold 10% $H_2SO_4$, most of the methanol was evaporated, and the product was extracted with ethyl acetate (5 ml) in three portions. The extract was washed with water (twice) and the solvent was evaporated. Chromatography of the residue on silica gel column (10 g) in a mixture of chloroform–methanol (20 : 1) with 0.5% of acetic acid gave 60 mg (70%) of glassy acid **16**. IR spectrum (KBr): 3 360 (N–H); 3 290 sh (O–H); 2 641, 2 585, 2 544 (COOH, dimer); 1 729 (C=O, COOH); 1 705 (C=O, ketone); 1 653, 1 525 (CONH); 1 052 (C–O). $^1$H NMR spectrum (500 MHz): 6.66 bd, 1 H, $J$ = 8.4 (NH); 4.19 dddd, 1 H, $J$ = 8.8, 5.8, 3.7, 8.4 (H-15α); 3.63 t, 1 H, $J$ = 8.8 (H-17α); 2.40–2.80 bm, 5 H ($CH_2CH_2$ succinate + H-16α); 1.07 s, 3 H (3 × H-19); 0.95 s, 3 H (3 × H-18). For $C_{23}H_{35}NO_5$ (405.5) calculated: 68.12% C, 8.70% H, 3.45% N; found: 68.29% C, 8.90% H, 3.42% N.

*The authors are indebted Dr L. Bednarova and Dr P. Fiedler for taking and interpretation of IR spectra. Our thanks are due to Mrs M. Snopkova for measurements of 200 MHz $^1$H NMR and to Dr M. Budesinsky for measurements and interpretation of 500 MHz $^1$H NMR spectra. The elemental analyses were carried out in the Analytical Laboratory of this Institute (Dr V. Pechanec, Head).*

## REFERENCES

1. Cerny I., Slavikova T., Pouzar V.: Collect. Czech. Chem. Commun. *62*, 1642 (1997).
2. Boyer J. H.: J. Am. Chem. Soc. *73*, 5248 (1951).
3. Dupret J., Grenot C., Rolland de Ravel M., Kappus E., Cuilleron C. Y.: J. Steroid Biochem. *20*, 479 (1984).
4. Kelly R. W., Sykes P. J.: J. Chem. Soc. C *1968*, 416.
5. Matsui M., Kinuyama Y.: J. Chem. Soc., Perkin Trans. 1 *1976*, 1429.
6. Nose A., Kudo T.: Chem. Pharm. Bull. 36, 1529 (1988); Hashimoto H., Araki K., Miyzawa K.-I.: Carbohydr. Res. *99*, 59 (1982).
7. Yatsimirskaya E. A., Gavrilova E. M., Egorov A. M., Levashov A. V.: Steroids *58*, 547 (1993).
8. Fields R.: Biochem. J. *124*, 581 (1971).
9. Bolander F. F., Fellows R. E.: Biochemistry *14*, 2938 (1975).
10. Lapcik O., Hampl R., Hill M., Starka L., Kasal A., Pouzar V., Putz Z.: Collect. Czech. Chem. Commun. *61*, 799 (1996).