ON STEROIDS. CLII.*
MICROBIAL OXYGENATION OF B-NORSTEROIDS

J.Joska, Ž.Procházka, J.Fajkoš and F.Šorm

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6

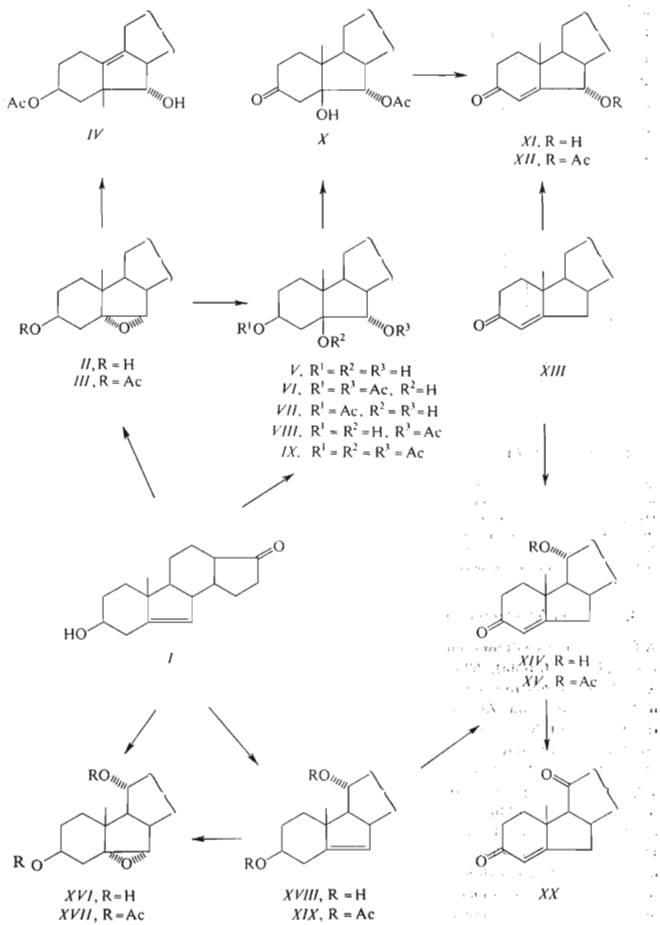
Received August 21st, 1972

Microbial oxygenation of 3β-hydroxy-B-norandrost-5-en-17-one with *Rhizopus nigricans* and of B-norandrost-4-en-3,17-dione with *Absidia orchidis* have been studied and the structures of the products isolated were established by independent syntheses.

In a previous communication[1] we have reported on microbial oxygenation of 3β-hydroxy-B-norandrost-5-en-17-one (*I*) with *Rhizopus nigricans* and of B-norandrost-4-en-3,17-dione with *Absidia orchidis*. The structures of the products obtained were assigned tentatively or left uncertain. In this paper we wish to report the detailed experimental work and to present chemical proofs of the structures of the oxygenated products.

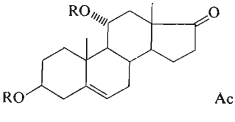
Microbial oxygenation of ketone[2] *I* with *R. nigricans* gave four main products: The epoxide *II*, the triol *V*, and the 11-oxygenated derivatives *XVI* and *XVIII*. The structure of the epoxide *II* has already been elucidated by our previous work[3]. When its acetate *III* was exposed to perchloric acid in acetone next to the product of Wagner–Meerwein rearrangement[3] the triol-monoacetate *VII* was isolated in about 70% yield. Hydrolysis led to the corresponding triol *V* identical in all respects with the second product of the microbial oxygenation. The configurations of the hydroxyl groups at $C_{(5)}$ and $C_{(6)}$ follow from our recent study[4] in the B-norcholestane series and are therefore opposite to those originally[1] assigned. To the third product the structure of the 11α-oxygenated derivative *XVIII* was assigned as the most probable. This structure has now been proved as follows: The known[5,8] 11α-hydroxy derivative *XXI* was transformed to the acetate *XXII* and the B-ring was contracted by the standard method[6] *via* the lactone *XXIV* to the corresponding B-norsteroid *XIX*, identical with the acetate prepared from the hydroxylated product *XVIII*. The fourth product of microbial transformation was acetylated to the diacetate and proved to be identical with the epoxide obtained from the diacetate *XIX* with peracid. As the fact that the peracid epoxidation of the 5,6-double bond in B-norsteroids proceeds from the α-face of the molecule is well established[3] this fourth product of hydroxylation is the diol-epoxide *XVI*.

* Part CLI: This Journal *38*, 913 (1973).

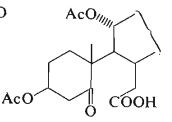


In further experiments the dione[7] *XIII* was oxygenated with *A. orchidis* under similar conditions. In this case two isomeric mono-hydroxylated products were

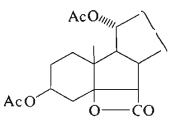
isolated. One of these compounds was prepared by Oppenauer oxidation from the diol *XVIII* and is therefore the 11α-hydroxy derivative *XIV*; it was also oxidised to the trione *XX*. The second product was prepared from the monoacetate *VIII* obtained on partial hydrolysis of the diacetate *VI*. It was oxidised to the ketone *X* and the 5β-hydroxyl was eliminated[4] with base or acetic acid to the unsaturated alcohol *XI* or acetate *XII*. The alcohol was identical with the second oxygenation product which is therefore the 6α-hydroxy derivative *XI*.



XXI, R = H
XXII, R = Ac



XXIII



XXIV

EXPERIMENTAL

Melting points were determined on a Kofler block. Analytical samples were dried at 80°C/0·2 Torr. Optical measurements were carried out in chloroform with an error of ±1°. The infrared spectra were recorded on the Zeiss UR 10 spectrometer. UV spectra were recorded on the CF 4 spectrometer in ethanol. The identity of samples prepared by different routes was checked by mixture melting point determination, by thin-layer chromatography, and by infrared spectra. Ligroin of b.p. 40—60°C was used as solvent. Working up of an ethereal solution means extraction with 5% HCl water, 5% $NaHCO_3$ and water, drying with magnesium sulphate, and evaporation of the solvent.

3β-Hydroxy-5,6α-epoxy-5α-B-norandrostan-17-one (*II*)

A fermentation medium composed of 200 g of bactopeptone (Organopharma), 1 000 g of apyrogenic glucose (Spofa), 60 g of corn steep oil, 20 g of ammonium nitrate and water up to 20 l was sterilised and inoculated with 1 l suspension of a 48 h old culture of *Rhizopus nigricans* in the same medium. Aeration and stirring was set on and the mold allowed to grow at 28°C for 24 h. A solution of the ketone[2] *I* (10 g) in methanol (100 ml) was then added under sterile conditions to the above culture and fermented for 28 h. The filtrate was extracted with chloroform (4 . 10 l), the combined extracts were washed twice with 5% $NaHCO_3$ (2 . 2 l), dried over sodium sulphate, and evaporated to dryness under reduced pressure. The residue was extracted with five 50 ml portions of ligroin, and the extract re-extracted with 50 ml of 90% methanol. The ligroin residue was discarded and the methanolic extract analysed chromatographically (Whatman No 4 paper impregnated with formamide, benzene as the mobile phase, detection with $SbCl_3$ in chloroform). The extract was dissolved in about 500 ml of benzene and chromatographed on an alumina column (act. III—IV; 500 g) collecting 1 liter fractions. The elution was started with benzene and continued with benzene–ether mixtures (20 : 1 for fractions 15—19, 10 : 1 for fractions 20—40, 3 : 1 for fractions 41—47) and eventually with pure ether. Fractions 15—19 contained 390 mg of the unreacted starting material. Fractions 21—31 were combined, evaporated, and the residue was crystallised from ethyl acetate to yield 1·51 g of the epoxide *II*, m.p. 195 to 198°C, $[\alpha]_D^{20}$ +8° (*c* 1·18) identical with the authentic[3] sample.

3β-Acetoxy-5β-methyl-6α-hydroxy-19,B-bisnorandrost-9-en-17-one (*IV*)

A solution of the epoxide *III* (3 g) in acetone (120 ml) was treated at room temperature with diluted perchloric acid (2·5 ml of 70% acid and 6 ml of water) and allowed to stand for 80 minutes at room temperature. The reaction mixture was poured into 5% $NaHCO_3$ (300 ml) and the product taken into ethyl acetate. The organic layer was washed with water, dried, and evaporated. The residue, containing accordiny to TLC next to the starting material two new polar compounds, was chromatographed on a silica gel column (150 g) in benzene–ether (1 : 1) 5 ml fractions being taken. Fractions 29—50 afforded 220 mg of the starting epoxide. Fractions 105—189 contained the less polar cleavage product. Combination and evaporation left 730 mg of a product which on crystallisation from methanol yielded 600 mg of the alcohol *IV*, m.p. 99—101°C, $[\alpha]_D^{20}$ +127° (*c* 1·76) identical with the authentic[3] sample.

3β,5β,6α-Trihydroxy-5β-B-norandrostan-17-one (*V*)

a) *From* 3β-*hydroxy*-B-*norandrost-5-en*-17-*one* (I) *by microbial oxygenation:* Fractions 83—108 from the chromatography after isolation of the epoxide *II* contained the most polar component. Combination and evaporation gave 1·7 g of a product which after crystallisation from ethyl acetate yielded 1·34 g of the triol *V*, m.p. 172—174°C, $[\alpha]_D^{20}$ +61° (*c* 2·73). For $C_{18}H_{28}O_4$ (308·4) calculated: 70·10% C, 9·15% H; found: 70·03% C, 9·00% H.

b) *From* 3β-*acetoxy*-5β,6α-*dihydroxy*-5β-B-*norandrostan*-17-*one* (VII): A solution of *VII* (200 mg) in methanol (2 ml) was treated with a solution of potassium hydroxide (200 mg) in the same solvent (1 ml) and allowed to stand at 30°C for 30 min. The reaction mixture was diluted with water and the product extracted into ethyl acetate. The extract was washed with water, dried, and the solvent distilled off under reduced pressure. The residue was crystallised from ethyl acetate–ligroin to yield 125 mg of the triol *V*, m.p. 175—177°C, $[\alpha]_D^{20}$ +62° (*c* 1·27), identical with the sample prepared as under *a*).

3β,6α-Diacetoxy-5β-hydroxy-5β-B-norandrostan-17-one (*VI*)

A) The triol *V* (368 mg) was acetylated with acetic anhydride (2 ml) in pyridine (3 ml) for 18 h at room temperature. The reaction mixture was decomposed with ice, the product isolated with ether, and the ethereal solution worked up. The product after evaporation of the solvent crystallised from ligroin and twice from diisopropyl ether to yield 121 mg of the diacetate *VI*, m.p. 110—112°C, $[\alpha]_D^{20}$ +58° (*c* 1·24). For $C_{22}H_{32}O_6$ (392·5) calculated: 67·32% C, 8·22% H; found: 67·55% C, 8·40% H.

B) The mono-acetate *VII* (250 mg) was acetylated with acetic anhydride (1·5 ml) in pyridine (2 ml) for 18 h at room temperature. Usual working up afforded a product which was chromatographed over silica gel (8 g) in benzene–ether (4 : 1). Working up of the corresponding fractions and crystallisation from ether–ligroin afforded 210 mg of the diacetate *VI*, m.p. 109—111°C, $[\alpha]_D^{20}$ +56° (*c* 1·39), identical with the same sample prepared as under *a*).

3β-Acetoxy-5β,6α-dihydroxy-5β-B-norandrostan-17-one (*VII*)

Elution of the chromatography after isolation of the derivative *IV* afforded fractions (195—380) with the polar component. Combination, evaporation, and crystallisation from ethyl acetate–ligroin yielded 2 g of the acetate *VII*, m.p. 183—184°C, $[\alpha]_D^{20}$ +53° (*c* 1·33). For $C_{20}H_{30}O_5$ (350·4) calculated: 68·54% C, 8·63% H; found: 68·31% C, 8·60% H.

6α-Acetoxy-3β,5β-dihydroxy-5β-B-norandrostan-17-one (*VIII*)

A solution of the diacetate *VI* (2·57 g) in methanol (150 ml) was treated with a solution of potassium carbonate (1·92 g) in water (28 ml) and kept at 50°C for 12 min. The reaction mixture was diluted with water, the product extracted with ether, the ethereal solution was washed with water, dried, and evaporated. The residue was chromatographed on a silica gel column (120 g) in benzene–ether (1 : 1). The corresponding fractions were combined, evaporated, and the residue was crystallised from ethyl acetate–ligroin to yield 1·65 g of the diol *VIII*, m.p. 102—104°C, $[\alpha]_D^{20}$ +35° (*c* 1·59). For $C_{20}H_{30}O_5$ (350·4) calculated: 68·54% C, 8·63% H; found: 68·29% C, 8·50% H.

3β,5β,6α-Triacetoxy-5β-B-norandrostan-17-one (*IX*)

The diacetate *VI* (291 mg) in acetic anhydride (5 ml) was treated with *p*-toluenesulphonic acid (91 mg) and heated to 100°C for 35 min. The reaction mixture was decomposed with ice, the product taken into ether, and the ethereal solution was worked up. The residue was crystallised from aqueous methanol to yield 190 mg of the triacetate *IX*, m.p. 169—171°C, $[\alpha]_D^{20}$ +87° (*c* 1·29). For $C_{24}H_{32}O_7$ (432·5) calculated: 66·65% C, 7·46% H; found: 66·36% C, 7·76% H.

6α-Acetoxy-5β-hydroxy-5β-B-norandrostan-3,17-dione (*X*)

The alcohol *VIII* (780 mg) in acetone (35 ml) was treated with excess Jones' reagent and allowed to stand for 10 minutes. The excess oxidising agent was removed with methanol, the reaction mixture diluted with water, and the product extracted with ethyl acetate. The extract was washed with a $NaHCO_3$ solution, water, dried, and evaporated. The residue was chromatographed on a silica gel column (40 g) in benzene–ether (1 : 1). Working up of the corresponding fractions and crystallisation from ether–ligroin gave 610 mg of the dione *X*, m.p. 156—158°C, $[\alpha]_D^{20}$ +100° (*c* 1·26). For $C_{20}H_{28}O_5$ (348·4) calculated: 68·94% C, 8·10% H; found: 68·79% C, 8·02% H.

6α-Hydroxy-B-norandrost-4-en-3,17-dione (*XI*)

a) *From* B-*norandrost*-4-*en*-3,17-*dione* (XIII) *by microbial oxygenation*: Identical fermentation medium as described above for the oxygenation of the alcohol *I* (2 liters) was sterilised and inoculated with the spores of *Absidia orchidis* and cultivated for 48 h at 28°C on a reciprocal shaker in nine 500 ml flasks. The dione[7] *XIII* (1 g) in methanol (20 ml) was then added to the well grown culture under sterile conditions, and shaking was continued for another 48 h. The mycelium was filtered off, washed with chloroform, and the filtrate extracted four times with 750 ml portions of chloroform. The combined extracts were washed with sodium hydrogen carbonate, water, and evaporated, yielding 925 mg of a semicrystalline solid which was combined with a similar material from other, preliminary experiments. The total (1·24 g) was dissolved in a small amount of benzene and applied on the top of a column of Hyflo-Supercel impregnated with formamide (450 ml of freshly distilled formamide per 500 g of the carrier; 650 g of this material were filled into a chromatographic tube containing benzene, pressing the adsorbent with a packing rod; column diameter 4·6 cm, length 69 cm). Elution was carried out with benzene and fractions of 250 ml were collected after 1100 ml of the eluate were taken separately containing the starting material and lipophilic impurities. Fractions 10—19 containing 501 mg of a uniform product were combined, evaporated, and the residue was crystallised from benzene. Yield 356 mg of the alcohol *XI*, m.p. 210—213°C, $[\alpha]_D^{20}$ +57·5° (*c* 2·45); UV: λ_{max} 238 nm (log ε 4·36), 300 (1·66); IR: 1667, 3450, 3616 cm^{-1}. For $C_{18}H_{24}O_3$ (288·4) calculated: 74·97% C, 8·38% H; found: 74·81% C, 8·27% H.

b) *From* 6α-*acetoxy*-5β-*hydroxy*-5β-B-*norandrostan*-3,17-*dione* (X): A solution of the acetate *X* (100 mg) in methanol (4 ml) was heated to 50°C in a nitrogen atmosphere with a solution of po-

tassium hydroxide (300 mg) in methanol (6 ml) for 20 min. The cooled mixture was treated with acetic acid (2 ml), diluted with water and the product extracted into ethyl acetate. The organic layer washed with a $NaHCO_3$ solution, water, dried, and evaporated. The residue was chromatographed on a silica gel column (5 g) in benzene–ether (1 : 1). Fractions containing the desired product were combined, evaporated, and the residue was crystallised from ethyl acetate to yield 35 mg of the alcohol *XI*, m.p. 215—217°C, $[\alpha]_D^{20}$ +59° (*c* 1·29), identical with the sample prepared as under *A*).

6α-Acetoxy-B-norandrost-4-en-3,17-dione (*XII*)

A) The alcohol *XI* (127 mg) was acetylated with acetic anhydride (1 ml) in pyridine (1·3 ml) at room temperature for 18 h. Usual working up and crystallisation from methanol afforded 107 mg of the acetate *XII*, m.p. 173—175°C, $[\alpha]_D^{20}$ −73° (*c* 2·16). For $C_{20}H_{26}O_4$ (330·4) calculated: 72·70% C, 7·93% H; found: 72·61% C, 7·82% H.

B) A solution of the dione *X* (200 mg) in acetic acid (7 ml) was refluxed under nitrogen for 3 h. The reaction mixture was cooled, diluted with water, the product taken into ethyl acetate, and the organic layer was washed with 5% $NaHCO_3$, water, dried, and evaporated. The residue was crystallised from methanol to yield 110 mg of the acetate *XII*, m.p. 177—178°C, $[\alpha]_D^{20}$ −72° (*c* 1·56), identical with the sample prepared as under *a*).

11α-Hydroxy-B-norandrost-4-en-3,17-dione (*XIV*)

a) *From* B-*norandrost*-4-*en*-3,17-*dione* (XIII) *by microbial oxygenation*: Fractions 20—23 from the chromatography after isolation of *XI* were combined, and evaporated to leave 94 mg of a product. This material was separated on four 20 cm broad sheets of formamide impregnated Whatman No 4 paper with benzene. The zones containing the hydroxylation product of higher polarity was extracted with chloroform, and after washing the extract with water, it was evaporated to dryness. The residue (9 mg) was combined with the product obtained from fractions 24—33 (92 mg) and the total was chromatographed on 3 g of alumina (act. III—IV, neutral) in benzene–ether (10 : 1). Fractions containing the polar hydroxylation product were combined, evaporated, and the residue was crystallised from ethyl acetate to yield 42 mg of the alcohol *XIV*, m.p. 185—190°C, with recrystallisation at 90—110°C, $[\alpha]_D^{20}$ +23° (*c* 2·28). For $C_{18}H_{24}O_3$ (288·4) calculated: 74·97% C, 8·39% H; found: 74·88% C, 8·47% H.

b) *From* 3β,11α-*dihydroxy*-B-*norandrost*-5-*en*-17-*one* (XVIII): The diol *XVIII* (660 mg) was dissolved in toluene (30 ml) and cyclohexanone (10 ml) and 8·5 ml of the mixture were distilled off. A solution of aluminium isopropylate (700 mg) in toluene (3·5 ml) was added to the mixture and 10 ml of the distillate were collected in the course of 30 min. The reaction mixture was poured on ice (150 g), acidified with 40 ml of 4% HCl and the product was taken into ether. The ethereal solution was washed with a sodium hydrogen carbonate solution, water, dried, and the solvent distilled off untill the product started to crystallise. The crystalls were collected (462·3 mg) and recrystallised from benzene–methanol and then twice from ethyl acetate to yield 280 mg of the dione *XIV*, m.p. 194—196°C (recrystallisation at 90—110°C), $[\alpha]_D^{20}$ +24° (*c* 1·49), identical with the sample prepared as under *A*).

11α-Acetoxy-B-norandrost-4-en-3,17-dione (*XV*)

The alcohol *XIV* (27 mg) was acetylated with acetic anhydride (0·2 ml) in pyridine (0·3 ml) at room temperature for 18 h. Working up and crystallisation from methanol gave 18 mg of the acetate *XV*, m.p. 166—167°C, $[\alpha]_D^{20}$ +30° (*c* 1·63). For $C_{20}H_{26}O_4$ (330·4) calculated: 72·70% C 7·93% H; found: 72·25% C, 7·82% H.

3β,11α-Dihydroxy-5,6α-epoxy-5α-B-norandrostan-17-one (*XVI*)

Fractions 68—82 from the chromatography after isolation of the epoxide *II* were combined, and evaporated to yield 624 mg of a product which on crystallisation from ethyl acetate gave 240 mg of the diol *XVI*, m.p. 235—240°C, $[\alpha]_D^{20}$ −17·7° (*c* 1·86, ethanol). For $C_{18}H_{26}O_4$ (306·4) calculated: 70·56% C, 8·55% H; found: 69·56% C, 8·24% H.

3β,11α-Diacetoxy-5,6α-epoxy-5α-B-norandrostane-17-one (*XVII*)

A) The diol *XVI* (174 mg) was acetylated with acetic anhydride (1 ml) in pyridine (1·5 ml) for 18 h at room temperature. Working up and crystallisation from methanol yielded 143 mg of the diacetate *XVII*, m.p. 190—193°C, $[\alpha]_D^{20}$ −16·6° (*c* 1·65); IR: 1411, 1257, 1738 cm^{-1}. For $C_{22}H_{30}O_6$ (390·5) calculated: 67·67% C, 7·74% H; found: 67·62% C, 7·73% H.

B) The olefin *XIX* (40 mg) in chloroform (2 ml) was treated with a solution of perphthalic acid (35 mg) in ether (0·5 ml) and allowed to stand at room temperature for 20 h. The reaction mixture was diluted with ether, the excess peracid extracted into 5% Na_2CO_3, the ethereal solution was washed with water, dried, and evaporated. The residue was crystallised from methanol to yield 28 mg of *XVII*, m.p. 190—192°C, $[\alpha]_D^{20}$ −24° (*c* 0·95), identical with the sample prepared as under *A*).

3β,11α-Dihydroxy-B-norandrost-5-en-17-one (*XVIII*)

a) *From* 3β-*hydroxy*-B-*norandrost*-5-*en*-17-*one by microbial oxygenation* (I): Fractions 50—61 from the chromatography after isolation of the epoxide *II* were combined and evaporated to yield 2·506 g of a semisolid product. Repeated crystallisation from ethyl acetate afforded 1·43 g of the diol *XVIII*, m.p. 114—120°C, $[\alpha]_D^{20}$ −85·5° (*c* 2·22 in ethanol). For $C_{18}H_{26}O_3$ (290·4) calculated: 74·44% C, 9·03% H; found: 74·21% C, 8·93% H.

b) *From* 3β,11α-*diacetoxy*-B-*norandrost*-5-*en*-17-*one* (XIX): The diacetate *XIX* (100 mg) in methanol (5 ml) was refluxed with a solution of potassium carbonate (200 mg) in water (5 ml) for 2 h. Methanol was distilled off under reduced pressure, the residue diluted with water, and the product extracted with ether–ethyl acetate. The extract was washed with water, dried, and evaporated. The crystallisation from ethyl acetate yielded 60 mg of the diol *XVIII*, m.p. 116—124°C, $[\alpha]_D^{20}$ −80° (*c* 1·14 in ethanol), identical with the sample prepared as under *A*).

3β,11α-Diacetoxy-B-norandrost-5-en-17-one (*XIX*)

A) The lactone *XXIV* (380 mg) was heated to 180—190°C for 10 min. After cooling to room temperature the solid was dissolved in benzene and chromatographed on a silica gel column (30 g) in benzene–ether (9 : 1). Working up of the corresponding fractions and crystallisation from methanol afforded 314 mg of the diacetate *XIX*, m.p. 188—189°C, $[\alpha]_D^{20}$ −79° (*c* 1·23). For $C_{22}H_{30}O_5$ (374·5) calculated: 70·56% C, 8·08% H; found: 70·50% C, 7·96% H.

B) The diol *XVIII* (40 mg) was acetylated with acetic anhydride (0·2 ml) in pyridine (0·3 ml) for 18 h at room temperature. Working up and crystallisation from methanol yielded 32 mg of the diacetate *XIX*, m.p. 184—186°C, $[\alpha]_D^{20}$ −75° (*c* 2·60), identical with the sample prepared as under *A*).

B-Norandrost-4-en-3,11,17-trione (*XX*)

The alcohol *XIV* (248 mg) in acetic acid (14 ml) was treated with a solution of chromic acid (74 mg) in 80% acetic acid (8 ml) and allowed to stand at room temperature for 20 h. The excess

chromic acid was removed with methanol and the organic solvents were distilled off under reduced pressure. The residue was treated with ether and water, the ethereal layer was separated, washed with ether, sodium hydrogen carbonate, water, dried, and evaporated. The residue was crystallised from methanol to yield 184 mg of the trione *XX*, m.p. 238—245°C, $[\alpha]_D^{20}$ +164° (*c* 2·73). For $C_{18}H_{22}O_3$ (286·4) calculated: 75·49% C, 7·75% H; found: 75·39% C, 7·79% H.

3β,11α-Diacetoxyandrost-5-en-17-one (*XXII*)

The diol *XXI* (6·6 g) was acetylated with acetic anhydride (40 ml) in pyridine (60 ml) for 20 h at room temperature. The reaction mixture was decomposed with ice, diluted with water, and the product collected by suction. It was dissolved in ether, the ethereal solution was worked up, and the product was crystallised from methanol. Yield 8·1 g of the diacetate *XXII*, m.p. 174 to 175°C, $[\alpha]_D^{20}$ −50° (*c* 1·32). For $C_{23}H_{32}O_5$ (388·5) calculated: 71·10% C, 8·30% H; found: 71·00% C, 8·21% H.

3β,11α-Diacetoxy-17-oxo-B-norandrostan-5ξ-ol-6-oic Acid, 5,6-Lactone (*XXIV*)

Chromic acid (6·5 g) in 50% acetic acid (15 ml) was added drop by drop within 1 hour and under vigorous stirring to a solution of the diacetate *XXII* (8 g) in acetic acid (80 ml) the temperature being kept at 55°C. The reaction mixture was stirred for another hour at the same temperature, methanol (15 ml) was then added to destroy the excess oxidising agent, and organic solvents were removed under reduced pressure. The residue was treated with water and ether, the ethereal solution was separated, washed with water, and the acidic products were extracted into 5% Na_2CO_3. The extract was acidified at 0°C with hydrochloric acid, the product extracted into ether, and the ethereal solution was washed with water, dried and evaporated, to leave 1·35 g of the acidic product. It was dried over P_2O_5, dissolved in pyridine (3·5 ml), treated with benzoyl chloride (1·1 ml), and allowed to stand at room temperature for 48 h. The reaction mixture was decomposed with ice, diluted with water, and the product taken into ether. The ethereal solution was worked up, and the residue was chromatographed on a silica gel column (50 g) in benzene–ether (4 : 1) containing 0·5% of pyridine. The corresponding fractions were combined, evaporated, and the residue was crystallised from methanol to yield 410 mg of the lactone *XXIV*, m.p. 141 to 145°C, $[\alpha]_D^{20}$ +25° (*c* 1·04). For $C_{23}H_{30}O_7$ (418·5) calculated: 66·01% C, 7·23% H; found: 65·89% C, 7·12% H.

The analyses were carried out in the Analytical Laboratory of this Institute by Mr V. Štěrba, Mrs E. Šípová and Mrs E. Sýkorová under the direction of Dr. J. Horáček. The IR and UV spectra were recorded by Mr P. Formánek under the direction of Dr J. Smolíková.

REFERENCES

1. Procházka Ž., Fajkoš J., Joska J., Šorm F.: This Journal *26*, 2068 (1961).
2. Joska J., Šorm F.: This Journal *23*, 1377 (1958).
3. Joska J., Fajkoš J., Šorm F.: This Journal *28*, 82 (1963).
4. Joska J., Fajkoš J., Šorm F.: This Journal *37*, 4091 (1972).
5. Raspe G., Richter H.: Chem. Abstr. *55*, P 13482 c (1961).
6. Šorm F., Dyková H.: This Journal *13*, 407 (1948).
7. Joska J., Fajkoš J., Šorm F.: This Journal *25*, 1086 (1960).
8. Procházka Ž., Buděšínský M., Prekajski P.: This Journal, in press.

Translated by the author (J.F.).