ALTERNATIVE SYNTHESES OF 2α,3α,17β-TRIHYDROXY--7-OXA-B-HOMO-5α-ANDROSTAN-6-ONE AND SOME ANDROSTANE BRASSINOLIDE ANALOGS*

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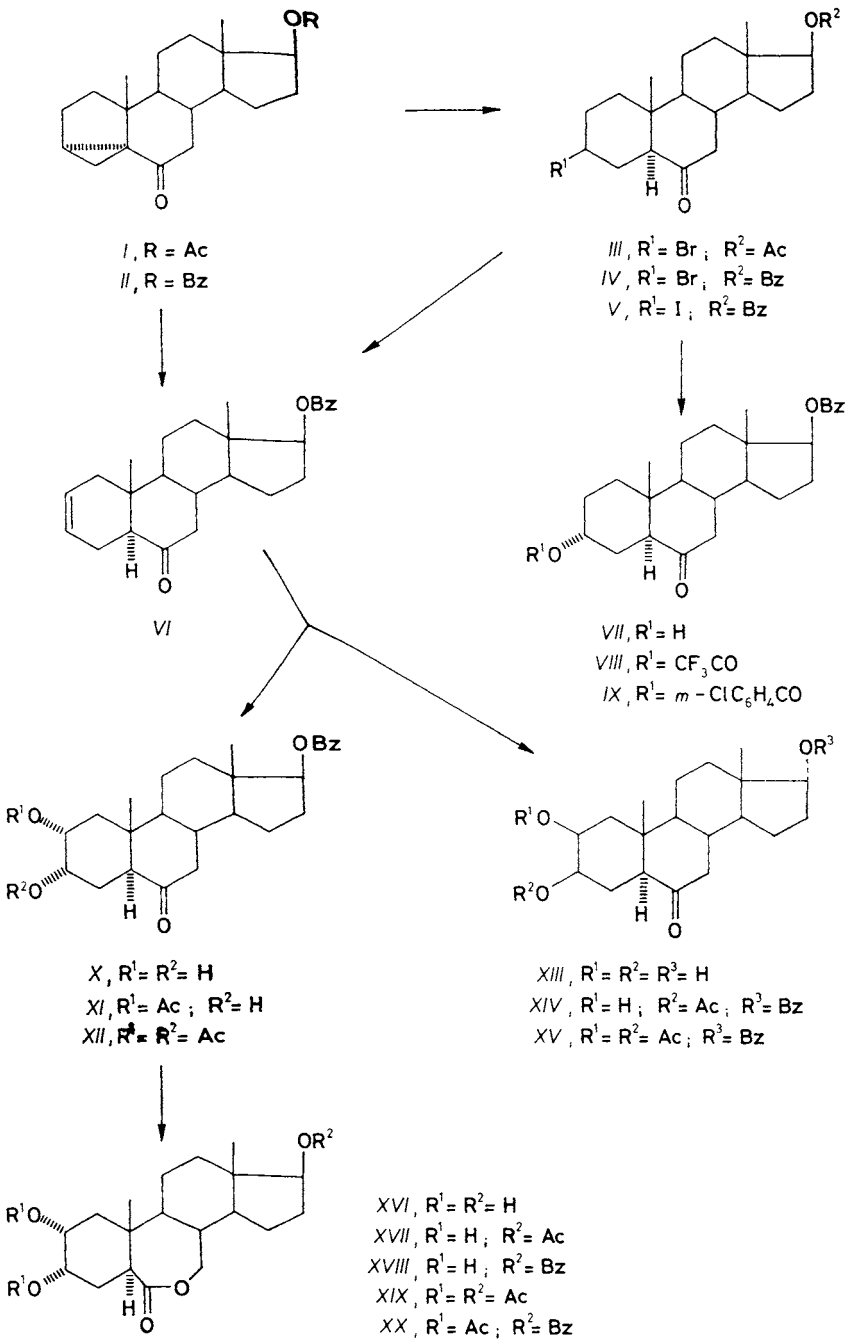
Received May 27th, 1986

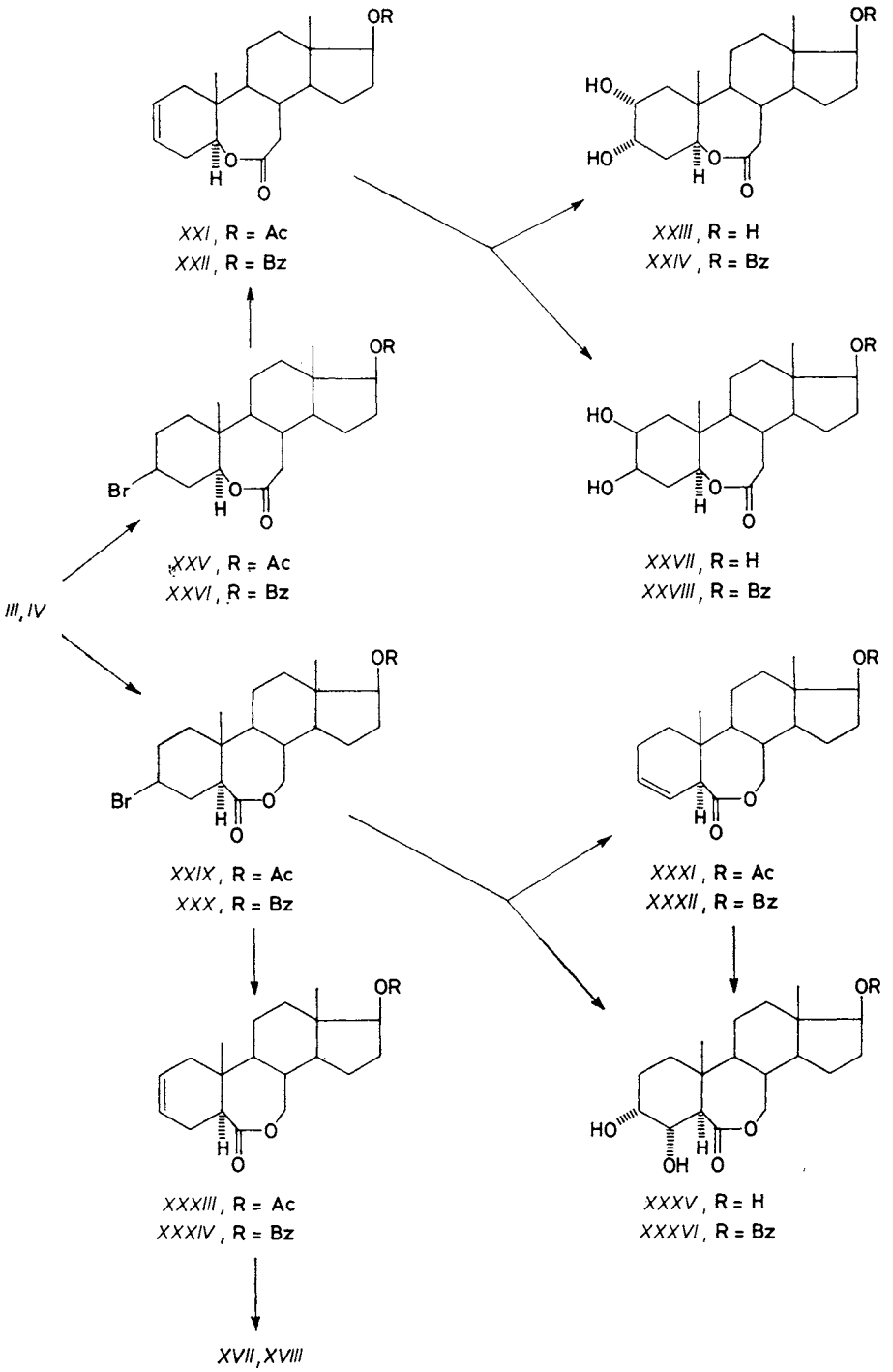
The title compound *XVI* was synthesized from *III*, *IV* or *V*. Related compounds *X*, *XIII*, *XVI*, *XVIII*, *XXIII*, *XXIV*, *XXVII*, *XXVIII*, *XXXV*, *XXXVI*, *XLI*, and *XLIII* were also prepared and tested by bean second internode bioassay. They showed only low activity (by about 2—3 orders or even lower than 24-epibrassinolide).

In the course of our investigation[1-7] on relationships between the structure and action of brassinolide analogs on plant growth, we needed steroids of the androstane type containing a lactonic B-ring, particularly derivatives of 2α,3α,17β-trihydroxy--7-oxa-B-homo-5α-androstan-6-one (*XVI*). At the time when our work was already in progress, Kondo and Mori[8] published a synthesis of the compound *XVI* by the application of a different approach. In the present paper, we publish our results representing an alternative synthesis of this triol. We also report on the syntheses of a series of its esters with organic acids including L-leucine and glycine together with the results of biologic testing of these derivatives and of some structurally modified analogs of *XVI*.

For the preparation of the triol *XVI* we set out from the 3β-bromo derivative *III* (ref.[9]), *IV* or from the 3β-iodo derivative *V*. We prepared the last two compounds from the 3α,5α-cyclosteroid *II* (ref.[10]) on treatment with the corresponding hydrogen halide. Starting from these compounds the sequence of the key operations may be: elimination of hydrogen halide to obtain the corresponding 2(3)-unsaturated derivative (*III*, *IV*, *V* → *VI*) followed by hydroxylation with OsO_4 (*X*) and formation of the lactonic moiety in the B-ring by means of Baeyer–Villiger oxidation (*XX*). Alternatively, the sequence may be: formation of the lactone grouping (*III* → *XXIX*, *IV* → *XXX*) followed by introduction of the 2(3)-double bond and osmium tetroxide hydroxylation. Baeyer–Villiger oxidation of the 6-ketones is known[11] to give rise

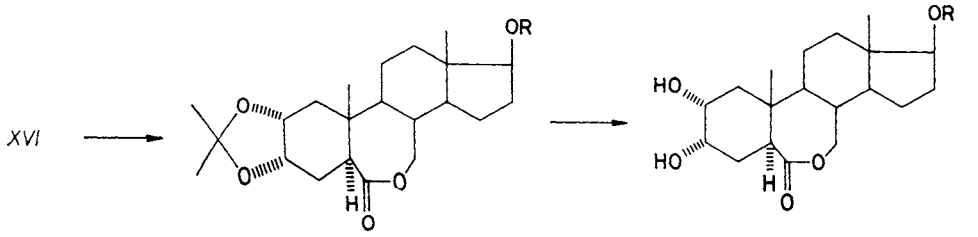
* Part CCCXXX in the series On Steroids; Part CCCXXIX: This Journal *52*, 775 (1987).


OR
O
I, R = Ac
II, R = Bz
OR²
R¹
H
O
III, R¹ = Br ; R² = Ac
IV, R¹ = Br ; R² = Bz
V, R¹ = I ; R² = Bz
OBz
H
O
VI
R¹O
VII, R¹ = H
VIII, R¹ = CF₃CO
IX, R¹ = m-ClC₆H₄CO
R²O
X, R¹ = R² = H
XI, R¹ = Ac ; R² = H
XII, R¹ = R² = Ac
OR³
XIII, R¹ = R² = R³ = H
XIV, R¹ = H ; R² = Ac ; R³ = Bz
XV, R¹ = R² = Ac ; R³ = Bz
XVI, R¹ = R² = H
XVII, R¹ = H ; R² = Ac
XVIII, R¹ = H ; R² = Bz
XIX, R¹ = R² = Ac
XX, R¹ = Ac ; R² = Bz


OR
O
H
XXI, R = Ac
XXII, R = Bz
HO
HO
XXIII, R = H
XXIV, R = Bz
Br
XXV, R = Ac
XXVI, R = Bz
XXVII, R = H
XXVIII, R = Bz
III, IV
XXIX, R = Ac
XXX, R = Bz
XXXI, R = Ac
XXXII, R = Bz
XXXIII, R = Ac
XXXIV, R = Bz
OH
XXXV, R = H
XXXVI, R = Bz
XVII, XVIII

to both regioisomeric lactones (7-oxa-6-oxo- and 6-oxa-7-oxo). Usually, the first structural type prevails but the ratio of both products depends on the structure of the ketone. In the case of the 3β-bromo derivative *III*, we have found the ratio of the products *XXIX* : *XXV* to be approximately 1 : 1. A similar mixture of the lactones *XXVI* and *XXX* is formed from *IV*, whereas oxidation of the compound *XII* gives 7-oxa--6-oxo derivative *XVII*. Therefore, the first sequence of steps appears more convenient. The action of peroxytrifluoroacetic acid on the iodo derivative *V* proceeds in a different manner: the major product of the reaction is the 3α-trifluoroacetate *VIII* along with the 3α-hydroxy derivative *VII*.

The elimination of hydrogen bromide from the lactonic 3β-bromo derivatives with LiBr–Li_2CO_3 in boiling dimethylformamide leads predominantly to a 2(3)-unsaturated derivative which is accompanied by a small amount of a 3(4)-olefin (*XXIX* → *XXXI*, *XXX* → *XXXII*). Osmium tetroxide hydroxylation of the 2(3)-unsaturated compounds (*VI*, *XXI*, *XXII*, *XXXIII*, *XXXIV*) led to the formation of 2α,3α-diols. In spite of the high stereoselectivity of the reagent approach to the steroid molecule, it was possible to isolate a small amount of the 2β,3β-diols in the case of the isomeric lactone *XXII* (→ *XXVIII*) and of the 6-ketone *VI* (→ *XIII*). On treatment with OsO_4, the 3(4)-unsaturated lactone *XXXII* provided the 3α,4α-diol *XXXVI* which was converted to the triol *XXXV*. The desired triol *XVI* was obtained by hydrolysis of the corresponding esters (*XVII*, *XIX*, *XX*). For bioassays, we also prepared 17-esters of *XVI* with L-leucine (*XLI*) and glycine (*XLIII*) starting from the isopropylidene derivative *XXXVII* via the esters with protected amino group (*XXXVIII*, *XL* and *XXXIX*, *XLII*).



XXXVII, R = H
XXXVIII, R = $COCH(NHZ)CH_2CH(CH_3)_2$
XXXIX, R = $COCH_2NHZ$

XL, R = $COCH(NHZ)CH_2CH(CH_3)_2$
XLI, R = $COCH(NH_2)CH_2CH(CH_3)_2$
XLII, R = $COCH_2NHZ$
XLIII, R = $COCH_2NH_2$

(Z = $COOCH_2C_6H_5$)

From the compounds described in this paper, the following ones were subjected to biologic testing: *X*, *XIII*, *XVI*, *XVIII*, *XXIII*, *XXIV*, *XXVII*, *XXVIII*, *XXXV*,

XXXVI, *XLI*, and *XLIV*. All the compounds showed only low activity. The compounds *XVIII* and *XXIV* are less active by about 2–3 orders, the compounds *X* and *XXXV* by about 3 orders and the remaining substances more than 3 orders less active than 24-epibrassinolide, *i.e.* (22*R*, 23*R*, 24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7--oxa-B-homo-5α-cholestan-6-one.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform with an error of ±3°. Unless stated otherwise, IR spectra were taken in tetrachloromethane on a Zeiss UR 20 spectrometer, ^{1}H NMR spectra on a Tesla B 476 (60 MHz) or Varian HA 100 (100 MHz) in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm and δ-scale. The symbol $W_{1/2}$ means half-height width of the signal. Interpretation of the spectra is based on first order analysis. The mass spectra were measured on an AEI MS 902 mass spectrometer. The identity of the samples prepared in various ways was checked by mixture melting point determination, thin-layer chromatography (TLC), IR and ^{1}H NMR spectra. Preparative TLC was conducted on 200 × 200 mm plates with a 0·7 mm thick silica gel layer. The term "usual workup" stands for washing the solution with 5% hydrochloric acid, water, 5% aqueous solution of potassium hydrogen carbonate, water, drying over sodium sulfate, filtration, and removing the solvent *in vacuo*. Petroleum ether used in the experiments boiled within the range of 40–62°C.

The bean second internode bioassay sensitized by Strnad *et al.*[12]: Bean seeds (*Phaseolus vulgaris* L., cv. PINTO) were germinated for two days and selected germinating seeds were transferred into pots containing perlite and grown[13] in Hoagland's solution diluted 1 : 10 from which nitrate was omitted and which was supplied with 3 mmol l^{-1} of Ca^{2+} and 0·1 mmol l^{-1} of Mn^{2+}. The pots were placed in a light-controlled cultivation room (25–27°C, light/dark period 16/8 h). The tested compounds were applied in fractionated lanoline as 1 μl microdrops to the base at the second internode (2–4 mm) of 7-day-old seedlings. The control plants were treated with lanoline alone. Results were expressed in mm of elongation after subtraction of the length of the control. The length of the second internode was measured 5 days after application of the test compounds. With respect to the double phasic response of internode elongation to brassinosteroids, the substances were tested over a broad range of doses and the evaluation was based on the first peak of activity.

3β-Bromo-17β-hydroxy-5α-androstan-6-one 17-Benzoate (*IV*)

17β-Hydroxy-3α,5-cyclo-5α-androstan-6-one 17-benzoate (*II*, 0·5 g) is dissolved in chloroform (1·5 ml) and acetic acid (5 ml) and a 30% solution of hydrobromic acid in acetic acid (1·5 ml) is added. After 30 min the mixture is diluted with water and the product taken up in ether, the solution washed with water, sodium hydrogen carbonate, water, dried with sodium sulfate and the solvent removed under reduced pressure. Crystallization from ethanol–acetone gives the product *IV* (432 mg), m.p. 208–210°C, $[\alpha]_D^{20}$ +10° (*c* 1·5). IR spectrum: 1 719, 1 275 (benzoate), 1 709 sh (C=O) cm^{-1}. Mass spectrum: *m/z* 472 + 474 (M). ^{1}H NMR spectrum: 0·80 (s, 19-H), 0·95 (s, 18-H), 3·86 (mt, $W_{1/2}$ = 22 Hz, 3α-H), 4·88 (mt, $W_{1/2}$ = 17 Hz, 17α-H), 7·30–7·73 and 7·85 to 8·18 (2 mt, benzoate protons). For $C_{26}H_{33}BrO_3$ (473·5) calculated: 65·96% C, 7·03% H, 16·88% Br; found: 66·08% C, 7·05% H, 16·91% Br.

3β-Iodo-17β-hydroxy-5α-androstan-6-one 17-Benzoate (*V*)

A solution of the ketone *III* (500 mg) in acetic acid (40 ml) is treated with aqueous hydriodic acid (1·2 ml, 67%) at room temperature overnight. After the addition of water, the mixture is extracted with chloroform, the organic phase washed with potassium hydrogen carbonate, water, dried, and the solvent evaporated. The residue is crystallized from ethyl acetate to give the iodo derivative *V* (360 mg), m.p. 203°C, $[\alpha]_D^{20}$ +27° (*c* 1·4). IR spectrum (chloroform): 1 712 (C=O), 1 712 and 1 280 (benzoate) cm^{-1}. ^1H NMR spectrum: 0·87 (s, 19-H), 0·95 (s, 18-H), 4·04 (mt, $W_{1/2}$ = = 19 Hz, 3α-H), 4·92 (dd, J = 7 Hz, J' = 10 Hz, 17α-H), 7·30–7·58 and 7·95–8·11 (2 mt, benzoate protons). For $C_{26}H_{33}IO_3$ (520·5) calculated: 60·00% C, 6·39% H, 24·38% I; found: 60·13% C, 6·11% H, 24·70% I.

17β-Hydroxy-5α-androst-2-en-6-one 17-Benzoate (*VI*)

a) *p*-Toluenesulfonic acid (40 mg) is added to a solution of the ketone *II* (733 mg) in dimethyl sulfoxide (5·3 ml) and the mixture heated under nitrogen at 160°C for 6 h, additional *p*-toluenesulfonic acid (80 mg) is added and heated for further 6 h. The mixture is poured into water, the product taken up in chloroform, the organic layer washed with water, potassium hydrogen carbonate, water, dried and the solvent removed under reduced pressure. The residue is chromatographed on a column of silica gel (100 g) in petroleum ether–ether (10 : 1) to give the product (560 mg). Crystallization from ethanol yields the olefin *VI* (255 mg), m.p. 215–218°C, $[\alpha]_D^{20}$ +36° (*c* 1·7). IR spectrum: 1 720, 1 275 (benzoate), 1 714 sh (C=O), 1 657 (C=C) cm^{-1}. ^1H NMR spectrum (100 MHz instrument): 0·74 (s, 19-H), 0·95 (s, 18-H), 4·94 (dd, J = 7·5 Hz, J' = 10 Hz, 17α-H), 5·47–5·87 (unresolved mt, 2-H and 3-H), 7·38–7·73 and 8·02–8·20 (2 mt, benzoate protons). For $C_{26}H_{32}O_3$ (392·5) calculated: 79·55% C, 8·22% H; found: 79·70% C, 8·09% H.

b) A mixture of the bromo derivative *IV* (75·2 g), lithium bromide (115 g) and lithium carbonate (12·7 g) is refluxed in dimethylformamide (850 ml) for 50 min. The mixture is poured on ice, the product separated with suction, taken up in benzene, the extract washed with 5% hydrochloric acid, water, sodium hydrogen carbonate, and filtered through a layer of silica gel. Evaporation leaves a crude product (54 g) which is chromatograhped on silica gel (1 000 g) in benzene–petroleum ether (25%). Crystallization of the purified fraction from benzene–ether gives the pure product (32·8 g), m.p. 217–218°C, $[\alpha]_D^{20}$ +31° (*c* 1·3). Found: 79·70% C, 8·09% H.

3α,17β-Dihydroxy-5α-androstan-6-one 17-Benzoate (*VII*)

a) A solution of the trifluoroacetate *VIII* (119 mg), chloroform (1·5 ml), methanol (4·5 ml) and hydrochloric acid (37%, 0·12 ml) is kept at room temperature for 48 h. The mixture is then poured into water and the product is extracted with chloroform. The extract is washed with water, potassium hydrogen carbonate, water, dried, and the solvent removed under reduced pressure. The residue (67 mg) is crystallized from methanol to give the 3α-alcohol *VII* (42 mg), m.p. 236–239°C, $[\alpha]_D^{20}$ +23° (*c* 1·1). IR spectrum: 3 625 (hydroxyl), 1 719, 1 274 (benzoate), 1 711 (C=O) cm^{-1}. Mass spectrum: *m/z* 410 (M), 395 (M − CH_3), 392 (M − H_2O), 377 (M − H_2O − CH_3), 288 (M − C_6H_5COOH; base peak), 273 (M − CH_3 − C_6H_5COOH), 270 (M − H_2O − C_6H_5·.COOH), 255 (M − CH_3 − H_2O − C_6H_5COOH). ^1H NMR spectrum: 0·75 (s, 19-H, 0·925 (s, 18-H), 4·15 (mt, $W_{1/2}$ = 6·5 Hz, 3β-H), 4·90 (dd, J = 6 Hz, J' = 9 Hz, 17α-H), 7·37–7·86 and 7·88–8·20 (2 mt, benzoate protons). For $C_{26}H_{34}O_4$ (410·5) calculated: 76·06% C, 8·35% H; found: 75·79% C, 8·14% H.

b) After isolation of the 3-chlorobenzoate *IX* (see preparation of *IX*) continuation of the chro-

matography gives a product (487 mg) which is crystallized from methanol to give the 3α-alcohol *VII* (185 mg), m.p. 234–236°C, $[\alpha]_D^{20}$ +23° (*c* 1·2).

3α,17β-Dihydroxy-5α-androstan-6-one 3-Trifluoroacetate 17-Benzoate (*VIII*)

Hydrogen peroxide (0·8 ml, 50%) is added to a solution of trifluoroacetic anhydride (3·76 ml) in dichloromethane (16 ml) and this solution is added to a mixture of the iodo derivative *V* (0·53 g), sodium hydrogen phosphate (3·18 g), and dichloromethane (32 ml). The mixture is refluxed while stirring under an atmosphere of nitrogen for two hours, then cooled, poured into 5% potassium hydrogen carbonate, the product extracted with chloroform, washed with water, dried with sodium sulfate, and the solvent evaporated under reduced pressure. The product is purified by preparative chromatography on 14 silica gel plates developed with petroleum ether–ether (3 : 2). The product (240 mg) is crystallized from a mixture of petroleum ether with ethylacetate to yield the trifluoroacetate *VIII* (165 mg), m.p. 181–183°C, $[\alpha]_D^{20}$ +20° (*c* 1·3). IR spectrum: 1 784, 1 225, 1 173, 1 147 (trifluoroacetate), 1 721, 1 274 (benzoate) cm^{-1}. Mass spectrum: *m/z* 506 (M), 491 (M – CH_3), 392 (M – CF_3COOH), 384 (M – C_6H_5COOH). For $C_{28}H_{33}O_5F_3$ (506·6) calculated: 66·39% C, 6·57% H, 11·25% F; found: 66·79% C, 6·39% H, 10·67% F.

3α,17β-Dihydroxy-5α-androstan-6-one 3-(3-Chlorobenzoate), 17-Benzoate (*IX*)

3-Chloroperoxybenzoic acid (1·2 g) is added to a solution of the iodo ketone *V* (1·2 g) in dichloromethane (22 ml) and the mixture let stand at room temperature for 20 min. The mixture is poured into water, the organic layer washed with water, potassium hydrogen carbonate, water, dried and the solvent removed under reduced pressure. The residue is chromatographed on silica gel (200 g); elution with benzene–ether (9 : 1) yields a lipophilic product (685 mg) which on crystallization from methanol gives 3-chlorobenzoate *IX* (370 mg), m.p. 225–227°C (sublimation from 210°C), $[\alpha]_D^{20}$ +23° (*c* 0·9). IR spectrum: 1 720 (C=O), 1 710, 1 274 (benzoate) cm^{-1}. Mass spectrum: *m/z* 408 (M – ClC_6H_4COOH; high resolution 408·2300, *i.e.* $C_{26}H_{32}O_4$ = M – ClC_6H_4·.COOH), 393 (408 – CH_3), 286 (408 – C_6H_5COOH), 271 (286 – CH_3), 156 (high resolution: 155·1143, *i.e.* $C_7H_5ClO_2$). ^{1}H NMR spectrum: 0·96 (s, 18-H and 19-H), 4·68–5·18 (complex mt, 3β-H and 17α-H), 7·36–7·65 and 7·88–8·19 (mts, benzoate protons). For $C_{33}H_{37}ClO_5$ (549·1) calculated: 72·18% C, 6·79% H, 6·46% Cl; found: 72·78% C, 6·43% H, 6·27% Cl.

2α,3α,17β-Trihydroxy-5α-androstan-6-one 17-Benzoate (*X*)

The compound *VI* (29 g) is dissolved in tetrahydrofuran (1 500 ml), diluted with water (300 ml), N-methylmorpholine N-oxide (40 g) and osmium tetroxide (1 g) are added. The mixture is stirred under argon at room temperature for 6 h, let stand overnight and after the addition of an aqueous (350 ml of water) solution of $Na_2SO_3.7\,H_2O$ stirred for 2 h. The product is taken up in dichloromethane (2 000 ml), the solution worked up as usual to yield the product (32 g), m.p. 243–245°C, which after crystallization from ethanol and benzene gives the diol *X* (14·5 g), m.p. 251–253°C. IR spectrum (chloroform): 3 615, 3 575 (hydroxyl), 1 710, 1 279 (C_6H_5COO), 1 710 (C=O) cm^{-1}. ^{1}H NMR spectrum: 0·76 (s, 19-H), 0·92 (s, 18-H), 3·72 (br mt, 2β-H), 4·01 (mt, $W_{1/2}$ = 8·5 Hz, 3β-H), 4·87 (mt, $W_{1/2}$ = 18 Hz, 17β-H), 7·33–7·60 and 7·89–8·17 (2 mt, benzoate protons). For $C_{26}H_{34}O_5$ (426·5) calculated: 73·21% C, 8·03% H; found: 73·12% C, 7·80% H.

2α,3α,17β-Trihydroxy-5α-androstan-6-one 2-Acetate, 17-Benzoate (*XI*)

Further continuation of the chromatography after isolation of the compound *XIV* yields 2α-monoacetate *XI* (111 mg), m.p. 264–266°C (subl. 250°C) (methanol), $[\alpha]_D^{20}$ 0°. IR spectrum:

3 605 (hydroxyl), 1 737, 1 254 (acetate), 1 711, 1 280 (benzoate) cm^{-1}. ^1H NMR spectrum: 0·84 (s, 19-H), 0·93 (s, 18-H), 4·10 (mt, $W_{1/2}$ = 8 Hz, 3β-H), 4·69—5·10 (mt, 2β-H and 17α-H), 7·33—7·59 and 7·86—8·12 (2 mt, phenyl protons). For $C_{28}H_{36}O_6$ (468·6) calculated: 71·77% C, 7·74% H; found: 71·62% C, 7·40% H.

2α,3α,17β-Trihydroxy-5α-androstan-6-one 2,3-Diacetate 17-Benzoate (*XII*)

a) The diol *X* (20 g) is acetylated at room temperature with acetic anhydride (100 ml) in pyridine (150 ml) for 48 h. The mixture is poured onto ice, filtered with suction, the cake washed with water, dissolved in benzene–ether, and worked up as usual. Crystallization from ethanol gives the product (20·8 g), m.p. 230—231°C, $[\alpha]_D^{20}$ +25° (*c* 1·4). IR spectrum: 1 749, 1 250 (acetate), 1 722, 1 277 (benzoate), 1 722 (C=O) cm^{-1}. Mass spectrum: *m/z* 510 (M), 450 (M — — CH_3COOH), 390 (M — 2 × CH_3COOH). ^1H NMR spectrum: 0·85 (s, 19-H), 0·93 (s, 18-H), 1·97 and 2·08 (2 s, 2α-acetate and 3α-acetate), 4·70—5·21 (2 overlapped mt, 2β-H and 17α-H), 5·38 (mt, $W_{1/2}$ = 5 Hz, 3β-H), 7·38—7·63 and 7·94—8·15 (2 mt, phenyl protons). For $C_{30}H_{38}O_7$ (510·6) calculated: 70·56% C, 7·50% H; found: 70·65% C, 7·38% H.

b) The monoacetate *XI* (100 mg) is acetylated as given under *a*). Usual workup and crystallization from methanol yields the diacetate *XII* (67 mg), m.p. 230—231°C.

c) The mother liquors (3 g) after crystallization of *X* are acetylated as under *a*). The mixture is chromatographed on silica gel (400 g) in petroleum ether–ether (7 : 3). Fractions containing the most lipophilic product yield the diacetate *XII* (1·94 g), m.p. 230—231°C, $[\alpha]_D^{20}$ +26°, (*c* 2·2).

2β,3β,17β-Trihydroxy-5α-androstan-6-one (*XIII*)

Aqueous potassium carbonate (50 mg in 1 ml) is added to a solution of the diacetate *XV* (50 mg) in methanol (10 ml) nad the mixture is kept at room temperature for 3 days. The mixture is concentrated under reduced pressure, diluted with water, the product taken up in ether, and the solution worked up as usual. The residue after evaporation is chromatographed on a silica gel plate and the plate developed with chloroform–ether (1 : 1). The major product is the more lipophilic 5α-triol *XIII* (27 mg), m.p. 235—238°C, $[\alpha]_D^{20}$ —3° (methanol, *c* 0·8) in agreement with literature[14].

2β,3β,17β-Trihydroxy-5α-androstan-6-one 3-Acetate 17-Benzoate (*XIV*)

Continued chromatography after separation of the compound *XV* (under *a*)) furnishes the 3β-acetate *XIV* (90 mg), m.p. 200—203°C (tetrachloromethane), $[\alpha]_D^{20}$ +44° (*c* 0·9). IR spectrum: 3 615, 3 515 (hydroxyl), 1 742 sh, 1 250 (acetate), 1 721, 1 276 (benzoate), 1 710 (C=O) cm^{-1}. ^1H NMR spectrum: 0·93 (s, 18-H), 1·02 (s, 19-H), 2·08 (s, 3-acetate), 4·08 (mt, $W_{1/2}$ = 7·5 Hz, 2α-H), 4·52—5·20 (complex mts, 2α-H and 17α-H), 7·37—7·63 and 7·92—8·19 (2 mt, phenyl protons). For $C_{28}H_{36}O_6$ (468·6) calculated: 71·77% C, 7·74% H; found: 71·34% C, 7·52% H.

2β,3β,17β-Trihydroxy-5α-androstan-6-one 2,3-Diacetate 17-Benzoate (*XV*)

a) From mother liquors after crystallization of *X* continued elution after separation of 2α,3α-diacetate *XII* as given under *c*) yields the 2β,3β-diacetate *XV* (109 mg), amounting to 58 mg, m.p. 235—236°C, $[\alpha]_D^{20}$ +28° (*c* 0·7) after crystallization from methanol. IR spectrum: 1 742, 1 249 (acetate), 1 723, 1 280 (benzoate), 1 710 (C=O) cm^{-1}. ^1H NMR spectrum: 0·92 (s, 18-H), 0·95 (s, 19-H), 1·99 and 2·06 (2 s, 2 × acetate), 4·83 (mt, $W_{1/2}$ = 19 Hz, 3α-H and 17α-H), 5·29 (mt, $W_{1/2}$ = 6 Hz, 2α-H), 7·36—7·63 and 7·93—8·15 (2 mt, phenyl protons). For $C_{30}H_{38}O_7$ (510·6) calculated: 70·56% C, 7·50% H; found: 70·43% C, 7·18% H.

b) A mixture of the monoacetate *XIV* (23 mg), pyridine (1 ml) and acetic anhydride (0·5 ml) is let stand at room temperature for 48 h. After dilution with ice water and extraction with ether, the solution is worked up as usual to give the oily product (25 mg) which is crystallized from methanol and furnishes the diacetate *XV* (9 mg), m.p. 233–235°C, $[\alpha]_D^{20}$ +26° (*c* 1·1).

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one (*XVI*)

a) The ester *XX* (10·1 g) is treated with aqueous (25 ml of water) potassium hydroxide (10 g) in methanol (700 ml) at room temperature for 3 days. The mixture is acidified (pH = 2·5) with hydrochloric acid and concentrated to about 1/5 of its volume, ethanol (200 ml) is added and concentrated to c. 150 ml. Potassium chloride precipitates and is filtered off, washed with ethanol and ether, the combined filtrates are concentrated almost to dryness and decanted with three 30 ml portions of ether to remove benzoic acid. The residue in the flask is dissolved in ethanol (250 ml), water (100 ml) is added and the solution concentrated to c. 100 ml. Repeated concentration gives several portions (amounting to 6 g) of the product, which after crystallization from aqueous ethanol yields the compound *XVI* (5·02 g), m.p. 213–214°C, $[\alpha]_D^{20}$ +46° (chloroform and a trace of ethanol, *c* 1·4). IR spectrum (KBr): 1 729, 1 711 sh (lactone), 3 450 (hydroxyls) cm^{-1}. For $C_{19}H_{30}O_5$ (338·4) calculated: 67·43% C, 8·94% H; found: 67·59% C, 8·67% H.

b) Potassium hydroxide (300 mg) is dissolved in water (1·75 ml) and added to a solution of the acetate *XVII* (500 mg) in methanol (40 ml). After standing at room temperature overnight, the mixture is acidified with hydrochloric acid, concentrated to 1/3 of the original volume and after the addition of water (2 ml), concentrated again. Addition of water and concentration is repeated, water is added (1/2 of the volume), and the crystalline product is collected on a Büchner funnel. It is recrystallized by dissolving in hot ethanol, the same amount of water is added, and the solution is concentrated on a rotary evaporator to incipient crystallization. After 2 h the crystals are collected by suction, washed with water to yield the product (340 mg), m.p. 212–213°C (opaque at 145°C), $[\alpha]_D^{20}$ +48° (chloroform with 2% of ethanol, *c* 1·3). Found: 67·54% C, 8·92% H.

c) The triacetyl derivative *XIX* (2·2 g) is suspended in methanol (180 ml) and benzene (10 ml) at 40°C, a solution of potassium hydroxide (2 g) in water (4 ml) is added and the mixture let stand overnight at room temperature. After acidification with hydrochloric acid the solution is concentrated and diluted with water. A part of the product crystallizes, is separated on a Büchner funnel and the filtrates are again concentrated and diluted with water. This procedure yields the product (1·4 g) which is purified by crystallization from aqueous ethanol to give the triol *XVI* (1·18 g), m.p. 212–214°C. Found: 67·35% C, 9·55% H.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Acetate (*XVII*)

The olefin *XXXIII* (2·8 g) is dissolved in tetrahydrofuran (150 ml), N-methylmorpholine N-oxide (4·2 g) in water (30 ml) and osmium tetroxide (100 mg) in tert-butanol (3 ml) are added. The mixture is stirred under argon at room temperature for 6 h, then let stand overnight. Sodium sulfite (7 g) in water (35 ml) is added, the mixture stirred for 3 h, and diluted with dichloromethane (450 ml) and water. The organic layer is washed with water several times, with 5% hydrochloric acid, water, sodium hydrogen carbonate and water. Evaporation under reduced pressure provides a residue (3·2 g) which is dissolved in a minimum of warm chloroform and the solution diluted with three volumes of warm ethanol. Approximately half of the solvent is distilled off, ethanol is added (half of the volume), and the mixture is allowed to crystallize. Repeated crystallization gives the product (2·66 g), m.p. 230–233°C, $[\alpha]_D^{20}$ +29° (*c* 0·14). IR spectrum (KBr): 1 728, 1 253, 1 196 (acetate), 1 715, 1 709 (lactone), 3 510, 3 385 (hydroxyl) cm^{-1}. For $C_{21}H_{32}O_6$ (380·5) calculated: 66·24% C, 8·48% H; found: 66·58% C, 8·38% H.

2α,3α,17α-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Benzoate (*XVIII*)

The olefin *XXXIV* (2·3 g) in acetone (120 ml) is hydroxylated by treatment with osmium tetroxide (115 mg) in tert-butanol (5·1 ml) in the presence of N-methylmorpholine N-oxide (2·3 g) dissolved in water (2·3 ml) at room temperature for 4 h. After workup analogous to that in the preceding experiment and crystallization from ethanol the product *XVIII* (2·02 g) melts at 245–247°C, $[\alpha]_D^{20}$ +76° (*c* 1·2). Mass spectrum: *m/z* 442 (M), 424 (M − H_2O), 406 (M − 2 × H_2O), 320 (M − C_6H_5COOH). IR spectrum (KBr): 3 510, 3 440 (hydroxyls), 1 712, 1 283 (benzoate), 1 712, 1 190, 1 141 sh (lactone) cm^{-1}. ^1H NMR spectrum (dimethyl sulfoxide): 0·90 (s, 18-H), 0·79 (s, 19-H), 2·95–4·44 (complex mt, 2α-H, 3β-H, 7a-H), 4·81 (br t, *J* = 8 Hz, 17α-H), 7·51 to 7·77 and 7·90–8·14 (2 mt, benzoate protons). For $C_{26}H_{34}O_6$ (442·5) calculated: 70·56% C, 7·74% H; found: 70·83% C, 7·72% H.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 2,3,17-Triacetate (*XIX*)

The monoacetate *XVII* (2·06 g) is acetylated with acetic anhydride (15 ml) in pyridine (40 ml) at room temperature overnight. After the usual workup, the product is filtered through silica gel in benzene–15% ether and crystallized from methanol to yield the triacetyl derivative *XIX* (2·2 g), m.p. 137–138°C, $[\alpha]_D^{20}$ +27° (*c* 1·6). IR spectrum: 1 745, 1 244, 1 052 (acetate), 1 745, 1 051, 1 025 (lactone) cm^{-1}. For $C_{25}H_{36}O_8$ (464·6) calculated: 64·63% C, 7·81% H; found: 64·74% C, 7·76% H.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 2,3-Diacetate 17-Benzoate (*XX*)

The ketone *XII* (27·5 g) is dissolved in dichloromethane (330 ml) and treated with a dichloromethane solution of pertrifluoroacetic acid prepared from $(CF_3CO)_2O$ (50 ml) and H_2O_2 (50%, 9 ml) in dichloromethane (740 ml) at 0°C. The solution is allowed to stand for 5 h, washed with H_2O, sodium thiosulfate, $NaHCO_3$, H_2O, dried and the solvent removed under reduced pressure. Chromatography on silica gel (900 g) in benzene–ether (2·5%) and crystallization from chloroform–ether yields pure *XX* (12·5 g), m.p. 262–263°C, $[\alpha]_D^{20}$ +73° (*c* 1·6). IR spectrum (chloroform): 1 738, 1 259 (acetate), 1 730 sh, 1 188, 1 072 (lactone), 1 712 sh, 1 280 (benzoate). For $C_{30}H_{38}O_8$ (526·6) calculated: 68·42% C, 7·27% H; found: 68·70% C, 7·21% H.

17β-Hydroxy-6-oxa-B-homo-5α-androst-2-en-7-one 17-Acetate (*XXI*)

The bromo derivative *XXV* (260 mg), lithium bromide (400 mg) and lithium carbonate (50 mg) in dimethylformamide (6 ml) are refluxed under argon for 50 min. The mixture is poured into water, taken up in ether, the ether solution washed with water, 5% hydrochloric acid, potassium hydrogen carbonate, and water, dried, and the solvent removed at reduced pressure. The residue is crystallized from ethanol (93 mg) m.p. 201–202°C, $[\alpha]_D^{20}$ +6° (*c* 1·3). For $C_{21}H_{30}O_4$ (346·5) calculated: 72·80% C, 8·73% H; found: 72·48% C, 8·76% H.

17β-Hydroxy-6-oxa-B-homo-5α-androst-2-en-7-one 17-Benzoate (*XXII*)

Reaction of the bromo derivative *XXVI* (78 mg), lithium bromide (40 mg), and lithium carbonate (40 mg) in dimethylformamide (1 ml) is conducted in the same manner as in the preceding experiment and furnishes a product (61 mg) which after crystallization from methanol yields the olefin *XXII* (20 mg), m.p. 233–235°C, $[\alpha]_D^{20}$ +51° (*c* 0·7). IR spectrum: 1 736 (lactone), 1 720, 1 274 (benzoate) cm^{-1}. Mass spectrum: *m/z* 408 (M). ^1H NMR spectrum: 0·95 (s, 18-H and 19-H), 2·32–2·60 (mt, 7a-H), 4·48 (t, *J* = 8 Hz, 5α-H), 4·86 (t, *J* = 8 Hz, 17α-H), 5·53 (d, *J* = 3 Hz,

2-H and 3-H), 7·34—7·60 and 7·89—8·15 (2 mt, benzoate protons). For $C_{26}H_{32}O_4$ (408·5) calculated: 76·44% C, 7·90% H; found: 76·40% C, 7·82% H.

2α,3α,17β-Trihydroxy-6-oxa-B-homo-5α-androstan-7-one (*XXIII*)

Aqueous potassium hydroxide (50%, 1·6 ml) is added to a solution of the benzoate *XXIV* (270 mg) in methanol (16 ml) and the mixture is refluxed for 1 h. After dilution with tetrahydrofuran (15 ml) and adicification with conc. hydrochloric acid refluxing is continued for 30 min. The solution is concentrated to dryness under reduced pressure, the residue extracted with chloroform, washed with water, dried and the solvent distilled off. The residue (170 mg) is crystallized from aqueous methanol to give the triol *XXIII* (65 mg), m.p. 299—301°C, $[\alpha]_D^{20}$ +14° (*c* 0·7). IR spectrum (KBr): 3 530, 3 505, 3 450, 1 072, 1 042 (hydroxyls), 1 725 sh, 1 701, 1 682, 1 281, 1 125 (lactone) cm^{-1}. ^1H NMR spectrum (mixture of C^2HCl_3 and $C^2H_3O^2H$; 100 MHz instrument): 0·75 (s, 18-H), 0·93 (s, 19-H), 2·40 (mt, 7a-H), 2·93—4·05 (mt, 2β-H and 17α-H), 4·52—4·74 (mt, 5α-H). For $C_{19}H_{30}O_5$ (338·4) calculated: 67·43% C, 8·94% H; found: 66·99% C, 8·91% H.

2α,3α,17β-Trihydroxy-6-oxa-B-homo-5α-androstan-7-one 17-Benzoate (*XXIV*)

Continued chromatography after separation of the compound *XXVIII* (see synthesis of the latter) yields the compound *XXIV* (911 mg), m.p. 290—292°C (subl.), $[\alpha]_D^{20}$ +49° (*c* 0·8). IR spectrum (KBr): 3 550, 3 390 (hydroxyls), 1 730, 1 040, 1 195 (lactone), 1 710, 1 280 (benzoate) cm^{-1}. Mass spectrum: *m/z* 442 (M), 424 (M − H_2O), 406 (M − 2 × H_2O), 395 (M − H_2O − CO, base peak), 320 (M − C_6H_5COOH). ^1H NMR spectrum: 0·93 (s, 18-H and 19-H), 3·33—4·12 (mt, 2β-H, 3β-H, and 5-H), 4·45—5·03 (mt, 17α-H), 7·30—7·70 and 7·93—8·22 (2 mt, benzoate protons). For $C_{26}H_{34}O_6$ (442·5) calculated: 70·56% C, 7·74% H; found: 70·15% C, 8·05% H.

3β-Bromo-17β-hydroxy-6-oxa-B-homo-5α-androstan-7-one 17-Acetate (*XXV*)

After separation of the compound *XXIX* (see preparation of the latter) the subsequent fractions give the second product (8 g) which after repeated crystallization from ethanol yields the pure *XXV* (3·7 g), m.p. 208—208·5°C, $[\alpha]_D^{20}$ +8° (*c* 1·4). IR spectrum (chloroform): 1 727, 1 256, 1 047 (acetate), 1 727 (lactone) cm^{-1}. ^1H NMR spectrum (200 MHz, C^2HCl_3): 0·808 (s, 18-H), 0·958 (s, 19-H), 2·042 (s, 17-acetate), 3·831 (m, $\sum J \approx 32{\cdot}9$ Hz, $J_{3\alpha,2\alpha} \approx J_{3\alpha,4\alpha} \approx 4{\cdot}1$, $J_{3\alpha,2\alpha} \approx J_{3\alpha,4\beta} \approx$ $\approx 12{\cdot}4$, 3α-H), 4·206 (dd, $J_{5\alpha,4\beta} \approx 11{\cdot}2$ Hz, $J_{5\alpha,4\alpha} \approx 5{\cdot}2$ Hz, 5α-H), 4·610 (dd, $J_{17\alpha,16\beta} =$ $= 7{\cdot}5$ Hz, $J_{17\alpha,16\alpha} = 9{\cdot}2$ Hz, 17α-H). For $C_{21}H_{31}BrO_4$ (427·4) calculated: 59·02% C, 7·31% H; 18·70% Br; found: 59·34% C, 7·40% H, 18·68% Br.

3β-Bromo-17β-hydroxy-6-oxa-B-homo-5α-androstan-7-one 17-Benzoate (*XXVI*)

a) The workup of the fractions containing the polar product in the preparation of the bromo lactone *XXX*, as given under *a*), yields the bromo lactone *XXVI* (98 mg), m.p. 283—286°C, $[\alpha]_D^{20}$ +50° (*c* 1·2). IR spectrum (chloroform): 1 724, 1 064, 1 050 (lactone), 1 712, 1 279 (benzoate) cm^{-1}. Mass spectrum: *m/z* 489 (M), 409 (M − Br), 366 (M − C_6H_5COOH). ^1H NMR spectrum: 0·95 (s, 18-H and 19-H), 2·35—2·58 (mt, 7a-H), 3·78 (mt, $W_{1/2} = 18$ Hz, 3α-H), 4·22 (dd, $J = 5{\cdot}5$ Hz, $J = 11$ Hz, 5α-H), 4·86 (mt, $W_{1/2} = 17$ Hz, 17α-H), 7·37—7·64 and 7·93 to 8·17 (2 mt, benzoate). For $C_{26}H_{33}BrO_4$ (489·5) calculated: 63·80% C, 6·80% H, 16·33% Br; found: 63·49% C, 6·82% H, 16·19% Br.

b) The workup of the zones containing the polar product as described in the preparation of the bromo lactone *XXX* (under *b*)) yields the bromo lactone *XXVI* (26 mg), m.p. 283—286°C, $[\alpha]_D^{20}$ +51° (*c* 1·1).

2β,3β,17β-Trihydroxy-6-oxa-B-homo-5α-androstan-7-one (*XXVII*)

A solution of the benzoate *XXVIII* (70 mg) in methanol (10 ml) is hydrolyzed with aqueous potassium hydroxide (50%, 1 ml) as described in the preparation of the compound *XXIII*. The crude product (65 mg) is crystallized from methanol to give the compound *XXVII* (18 mg), m.p. 277 to 278°C (chloroform). IR spectrum (KBr): 3 540, 3 515, 3 455, 1 059, 1 051 (OH), 1 740 sh, 1 713, 1 701, 1 311, 1 283 (CO) cm^{-1}. ^1H NMR spectrum (C^2HCl_3 + $C^2H_3O^2H$): 0·75 (s, 18-H), 0·95 (s, 19-H), 2·31—2·58 (mt, 7a-H), 3·31—4·51 (mt, 2α-H, 3α-H, 5α-H and 17α-H). For $C_{19}H_{30}O_5$ (338·4) calculated: 67·43% C, 8·94% H; found: 67·30% C, 8·71% H.

2β,3β,17β-Trihydroxy-6-oxa-B-homo-5α-androstan-7-one 17-Benzoate (*XXVIII*)

A solution of osmium tetroxide (70 mg) in tert-butanol (2 ml) and a solution of N-methylforpholine N-oxide (1·4 g) in water (2 ml) is added to a solution of the olefin *XXII* (1·4 g) in acetone (70 ml) and the mixture is stirred at room temperature for 4 h. After standing overnight, a 10% solution of sodium sulfite (10 ml) is added and the mixture is stirred for 30 min, the solution is poured into water and extracted with ether. After the usual workup, the residue (1·4 g) is chromatographed on silica gel (200 g) in chloroform–2-propanol (33 : 1). The workup of the fractions containing the less polar minor product gives the 2β,3β-diol *XXVIII* (356 mg) which after crystallization from 2-propanol melts at 294—296°C (subl., decomp.), $[\alpha]_D^{20}$ +25° (*c* 0·7). IR spectrum (KBr): 3 485, 3 410, 1 045 (hydroxyls), 1 730 sh, 1 195, 1 027 (lactone), 1 716, 1 286 (benzoate) cm^{-1}. Mass spectrum: *m/z* 442 (M), 424 (M − H_2O), 409 (M − H_2O − CH_3), 395 (M − − H_2O − CO), 320 (M − C_6H_5COOH, base peak). ^1H NMR spectrum: 0·95 (s, 18-H and 19-H), 3·68—4·54 (mt, 2α-H, 3α-H and 5α-H), 4·72—5·09 (mt, 17α-H), 7·40—7·67 and 7·94—8·26 (2 mt, benzoate protons). For $C_{26}H_{34}O_6$ (442·5) calculated: 70·56% C, 7·74% H; found: 70·43% C, 8·01% H.

3β-Bromo-17β-hydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Acetate (*XXIX*)

A solution of peroxytrifluoroacetic acid is prepared from hydrogen peroxide (85%, 5·72 ml) and trifluoroacetic anhydride (37 ml) in dichloromethane (572 ml) at 0°C. This reagent is added to a solution of the 6-ketone *III*, ref.[9] (14·9 g) in dichloromethane (200 ml) and left to stand at room temperature for 2 h. The solution is then washed with water, potassium hydrogen carbonate and water, dried over magnesium sulfate and the solvent evaporated under reduced pressure. TLC chromatography shows presence of two major products. Chromatography on silica gel (450 g) in benzene separates the first compound (3 g) which is still present in the intermediate fraction; rechromatography of the latter gives an additional 1·6 g of this product. Recrystallization of the combined crude crops from methanol gives the compound *XXIX* (3·5 g), m.p. 214—216°C, $[\alpha]_D^{20}$ +30° (*c* 1·3). IR spectrum (chloroform): 1 727, 1 256, 1 048 (acetate), 1 727, 1 184, 1 172, 1 162 (lactone) cm^{-1}. ^1H NMR spectrum (200 MHz, C^2HCl_3): 0·82 (s, 18-H), 0·95 (s, 19-H), 2·05 (s, 17-acetate), 2·86 (dd, $J_{5,4\alpha}$ = 4·4 Hz, $J_{5,4\beta}$ = 11·8 Hz, 5-H), 4·00 and 4·06 (AB part of ABX-system, m, $J_{7a\alpha,7a\beta}$ = 12·8 Hz, $J_{7a\alpha,8}$ = 9·8 Hz, $J_{7a\beta,8}$ = 1·5 Hz, 7aα-H and 7aβ-H), 4·60 (dd, 17-H). For $C_{21}H_{31}BrO_4$ (427·4) calculated: 59·02% C, 7·31% H, 18·70% Br; found: 59·39% C, 7·25% H, 18·47% Br.

3β-Bromo-17β-hydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Benzoate (*XXX*)

a) The ketone *IV* (230 mg) in dichloromethane (2·3 ml) is oxidized with peroxytrifluoroacetic acid in the same manner as in the preparation of the compound *XXIX*. After analogous workup the mixture is chromatographed on silica gel (100 g) in benzene–ether (49 : 1). The fractions con-

taining the lipophilic compound (55 mg) are crystallized from methanol to give the compound *XXX* (11 mg), m.p. 250–256°C, $[\alpha]_D^{20}$ +76° (*c* 0·4). IR spectrum (chloroform): 1 726, 1 185, 1 179 (lactone), 1 718, 1 281 (benzoate) cm^{-1}. Mass spectrum: *m/z* 488 (M), 408 (M – HBr), 366 (M – C_6H_5COOH). ^1H NMR spectrum: 0·94 (s, 18-H and 19-H), 3·68–4·27 (mt, 3α-H and 7a-H), 4·82 (mt, $W_{1/2}$ = 18 Hz, 17α-H), 7·34–7·61 and 7·88–8·12 (2 mt, benzoate protons). For $C_{26}H_{33}BrO_4$ (489·5) calculated: 63·80% C, 6·80% H, 16·33% Br; found: 63·75% C, 6·81% H, 16·20% Br.

b) A solution of the ketone *IV* (110 mg) and 3-chloroperbenzoic acid (110 mg) in dichloromethane (2 ml) is let stand at room temperature for 8 days. The mixture is poured into water, extracted with chloroform, the organic layer washed with water, potassium hydrogen carbonate, water, dried, and evaporated. The residue is composed of two compounds which are separated on 4 preparative plates (silica gel). The zones with the lipophilic component give the lactone *XXX* (26 mg), m.p. 250–255°C, $[\alpha]_D$ +72° (*c* 1·1).

17β-Hydroxy-7-oxa-B-homo-5α-androst-3-en-6-one 17-Acetate (*XXXI*)

Continuation of the chromatography after separation of the compound *XXXIII* gives an intermediate fraction (150 mg) and crystallizing oil as the minor component (100 mg) which on crystallization from ethanol provides the pure product *XXXI* (26 mg), m.p. 162–163°C, $[\alpha]_D^{20}$ ±0° (*c* 1·3). IR spectrum: 1 742, 1 246 (acetate), 1 742, 1 161 (lactone), 3 045, 1 671 (C=C) cm^{-1}. ^1H NMR spectrum (200 MHz, C^2HCl_3, tetramethylsilane); 0·84 (s, 18-H), 0·89 (s, 19-H), 2·05 (s, 17-acetate), 3·51 (m, $J_{5,4}$ = 2·0 Hz, $J_{5,3} \approx J_{5,2\alpha} \approx J_{5,2\beta} \approx$ 2·5 Hz, 5-H), 4·09 and 4·10 (AB part of ABX-system, $J_{7a\alpha,7a\beta}$ = 12·8 Hz, $J_{7a\alpha,8}$ = 11·4 Hz, $J_{7a\beta,8} \leqq$ 0·3 Hz, 7aα-H and 7aβ-H), 4·62 (dd, $J_{17,16\alpha}$ = 9·1 Hz, $J_{17,16\beta}$ = 7·5 Hz, 17-H), 5·58 (dq, $J_{4,3}$ = 10·3 Hz, $J_{4,5} \approx \approx J_{4,2\alpha} \approx J_{4,2\beta} \approx$ 2·0 Hz, 4-H), 5·92 (dm, $J_{3,4}$ = 10·2 Hz, 3-H). For $C_{21}H_{30}O_4$ (336·5) calculated: 72·80% C, 8·73% H; found: 72·29% C, 8·82% H.

17β-Hydroxy-7-oxa-B-homo-5α-androst-3-en-6-one 17-Benzoate (*XXXII*)

The zones with lipophilic component, as obtained on TLC separation of the compound *XXXIV*, are worked up to give the 3(4)-olefin *XXXII* (10 mg), m.p. 250–253°C, $[\alpha]_D^{20}$ +100° (*c* 0·7). IR spectrum: 3 035, 1 675 (C=C), 1 736, 1 179, 1 162 (lactone), 1 723, 1 276 (benzoate) cm^{-1}. Mass spectrum: *m/z* 408 (M). ^1H NMR spectrum: 0·90 and 0·98 (2 s, 18-H and 19-H), 3·52 (mt, $W_{1/2}$ = 9·5 Hz, 5α-H), 4·12 (d, *J* = 6 Hz, 7a-H), 4·88 (t, *J* = 8 Hz, 17α-H), 5·47–6·10 (mt, 3-H and 4-H), 7·33–7·65 and 7·96–8·19 (2 mt, benzoate protons). For $C_{26}H_{32}O_4$ (408·5) calculated: 76·44% C, 7·90% H; found: 76·38% C, 7·61% H.

17β-Hydroxy-7-oxa-B-homo-5α-androst-2-en-6-one 17-Acetate (*XXXIII*)

The bromo derivative *XXIX* (1·75 g), lithium bromide (2·7 g), and lithium carbonate (0·3 g) in dimethylformamide (20 ml) are refluxed for 50 min, poured into water and taken up in ether. The solution is worked up as usual to yield the product (1·62 g). TLC-chromatography reveals the presence of the major product accompanied by a small amount of the second component. Chromatography on silica gel (90 g) in benzene gives the major product (1·38 g) which after crystallization from ethanol yields the pure compound *XXXIII* (1·27 g), m.p. 168–169°C, $[\alpha]_D^{20}$ ±0° (*c* 1·3). IR spectrum: 1 741, 1 247 (acetate), 1 741, 1 161 (lactone), 3 035, 669 (C=C) cm^{-1}. ^1H NMR spectrum (200 MHz, C^2HCl_3): 0·83 (s, 18-H), 0·91 (s, 19-H), 2·05 (s, 17-acetate), 2·92 (m, 5-H), ~2·81 (m, 4-H), 4·06 and 4·07 (AB part of ABX-system, $J_{7a\alpha,7a\beta}$ = 12·6 Hz,

$J_{7a\alpha,8}$ = 10·3 Hz, $J_{7a\beta,8} \approx 0$ Hz, 7aα-H and 7aβ-H), 4·61 (dd, 17-H), 5·57 and 5·70 (2 mt, $J_{2,3}$ = 10·4 Hz, 2-H and 3-H). For $C_{21}H_{30}O_4$ (346·5) calculated: 72·80% C, 8·73% H; found: 72·48% C, 8·89% H.

17β-Hydroxy-7-oxa-B-homo-5α-androst-2-en-6-one 17-Benzoate (*XXXIV*)

Dehydrobromination of the bromo lactone *XXX* (69 mg) in the same manner as in the preparation of *XXXIII* provides an oily mixture of two components. Preparative TLC chromatography is conducted on 4 silica gel plates developed with benzene–ether 4 : 1. The zones with the lipophilic product provide the olefin *XXXIV* (42 mg), after crystallization from ethanol melting at 222 to 224°C, $[\alpha]_D^{20}$ +42° (*c* 1·3). IR spectrum: 3 030, 1 676 (C=C), 1 733 sh, 1 161 (lactone), 1 720, 1 276 (benzoate) cm^{-1}. Mass spectrum: *m/z* 408 (M), 286 (M − C_6H_5COOH), 271 (M − − C_6H_5COOH − CH_3). ^1H NMR spectrum: 0·92 and 0·98 (2 s, 18-H and 19-H), 2·86 (d, J = 9 Hz, 5α-H), 4·08 (d, J = 4·5 Hz, 7a-H), 4·86 (dd, J = 8 Hz, J' = 8 Hz, 17α-H), 3·62 (br s, $W_{1/2}$ = 5 Hz, 2-H and 3-H), 7·36–7·60 and 7·85–8·15 (2 mt, benzoate protons). For $C_{26}H_{32}O_4$ (408·5) calculated: 76·44% C, 7·90% H; found: 76·72% C, 7·80% H.

3α,4α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one (*XXXV*)

The benzoate *XXXVI* (340 mg) is hydrolyzed in the same manner as described above with *XXVII*. The residue (295 mg) is purified by preparative TLC using 8 silica gel plates developed with chloroform–methanol (9 : 1). The product (172 mg) is crystallized from chloroform to give the triol *XXXV* (95 mg), m.p. 247–249°C, $[\alpha]_D^{20}$ +13° (*c* 0·8). IR spectrum: 3 610, 3 585, 1 076 (hydroxyls), 1 712, 1 183 (lactone) cm^{-1}. ^1H NMR spectrum: 0·77 (s, 18-H), 0·92 (s, 19-H), 3·66 (dd, J = 7 Hz, J' = 7 Hz, 17α-H), 3·92–4·44 (mt, 3β-H, 4β-H, 7a-H). For $C_{19}H_{30}O_5$ (338·4) calculated: 67·43% C, 8·94% H; found: 67·11% C, 8·59% H.

3α,4α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Benzoate (*XXXVI*)

The olefin *XXXII* (1 g) is hydroxylated with osmium tetroxide (50 mg) and N-methylmorpholine N-oxide (1 g) as given in the preparation of the compound *XXVIII*. The workup gives a product which is crystallized from chloroform–methanol to give the compound *XXXVI* (600 mg), m.p. 233–235°C, $[\alpha]_D^{20}$ +82° (*c* 0·9). IR spectrum (chloroform): 3 590 (hydroxyls), 1 713, 1 281 (benzoate), 1 721, 1 179, 1 695 sh (lactone) cm^{-1}. Mass spectrum: *m/z* 442 (M), 424 (M − H_2O). ^1H NMR spectrum: 0·92 and 0·95 (2 s, 18-H and 19-H), 3·92–4·44 (mt, 3β-H, 4β-H and 7a-H), 4·81 (t, J = 8 Hz, 17α-H), 7·40–7·69 and 7·96–8·22 (2 mt, benzoate protons). For $C_{26}H_{34}O_6$ (442·5) calculated: 70·56% C, 7·74% H; found: 70·25% C, 7·76% H.

2α,3α-Isopropylidendioxy-17β-hydroxy-7-oxa-B-homo-5α-androstan-6-one (*XXXVII*)

The triol *XVI* (1·9 g) and anhydrous cupric sulfate (2 g) are suspended in acetone (40 ml) and a solution of 70% perchloric acid (0·4 ml) in acetone (15 ml) is added. The mixture is stirred for 2 h, then poured in aqueous sodium hydrogen carbonate, taken up in benzene–ether, the solution dried with magnesium sulfate and after the addition of two drops of pyridine the solvent is removed under reduced pressure. A drop of pyridine is added to the residue (2·13 g) and the latter is crystallized from aqueous acetone to give the product (1·38 g), m.p. 252–254°C, $[\alpha]_D^{20}$ +20° (*c* 1·5). IR spectrum (chloroform): 3 615 (hydroxyl), 1 729 (ketone), 1 261, 1 186, 1 074, 1 061, 902 (–O–: hydroxyl, lactone, ketal) cm^{-1}. For $C_{22}H_{34}O_5$ (378·5) calculated: 69·81% C, 9·05% H; found: 69·64% C, 8·84% H.

2α,3α-Isopropylidenedioxy-6-oxo-7-oxa-B-homo-5α-androstan-17β-ol 17-(N-Benzyloxycarbonyl-L-leucinate) (*XXXVIII*)

Dicyclohexylamine salt of N-benzyloxycarbonyl-L-leucine (1·35 g) in ethanol solution (50%, 60 ml) is stirred with Dowex 50 (H^+) suspension (15 ml) for 1 h, the resin is separated by suction, washed with ethanol (50%), the filtrate evaporated *in vacuo*, evaporation repeated three times after the addition of ethanol and toluene mixture, then twice with toluene. The oily residue is dissolved in toluene (30 ml), steroid *XXXVII* (567 mg, dried azeotropically with toluene), dicyclohexylcarbodiimide (250 mg) and 4-dimethylaminopyridine (30 mg) are added, and the mixture is stirred at room temperature. After 30 min, an additional dicyclohexylcarbodiimide (250 mg) is added, stirring continued for 15 min, and the mixture let stand overnight, filtered, the filtrate washed with H_2O, $NaHCO_3$, H_2O and the solvent evaporated under reduced pressure. The residue (1·15 g, foam) is chromatographed on aluminum oxide (III—IV, neutr. after Brockmann). Benzene–ether (20%) elutes the pure *XXXVIII* (0·95 g), $[\alpha]_D^{20}$ +17° (*c* 1·4). IR spectrum: 3 445, 1 730, 1 505 ($NHCOOCH_2C_6H_5$), 1 740 (lactone), 1 210, 1 184, 1 172, 1 071, 1 052 (—O—) cm^{-1}. For $C_{36}H_{51}NO_8$ (625·8) calculated: 69·09% C, 8·21% H, 2·24% N; found: 68·82% C, 8·15% H, 2·26% N.

2α,3α-Isopropylidenedioxy-7-oxa-6-oxo-B-homo-5α-androstan-17β-ol 17-(N-Benzyloxycarbonylglycinate) (*XXXIX*)

The steroid *XXXVII* (757 mg) (dried azeotropically with toluene) is dissolved in toluene (100 ml), the solution stirred magnetically, a suspension of N-benzyloxycarbonylglycine (732 mg, azeotropically dried) in toluene (150 ml) are delivered at 37°C and dicyclohexylcarbodiimide (1 g) and 4-dimethylaminopyridine (60 mg) is added in four portions at 12 h intervals; the temperature is kept at 37°C at the start, then allowed to become ambient. The suspended material is then filtered off, washed with benzene, the filtrate washed with H_2O, $NaHCO_3$, H_2O, dried, and the solvent evaporated. The residue is purified by chromatography on Al_2O_3 (neutr., III—IV, 25 g), in benzene–petroleum ether (3 : 1) and the oily residue crystallized from ether (1·13 g), m.p. 205—207°C and recrystallized from benzene–ether to give *XXXIX* (855 mg), m.p. 207—209°C, $[\alpha]_D^{20}$ +18° (*c* 1·3). For $C_{32}H_{43}NO_8$ (569·7) calculated: 67·47% C, 7·61% H, 2·46% N; found: 67·88% C, 7·67% H, 2·46% N.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-(N-Benzyloxycarbonyl-L-leucinate) (*XL*)

The isopropylidene derivative *XXXVIII* (900 mg) is dissolved in methanol (10 ml), and after the addition of conc. HCl (0·15 ml) the mixture is kept at room temperature for 90 min. Solid $NaHCO_3$ is added, the mixture diluted with aqueous $NaHCO_3$, the precipitate extracted with ether, washed with $NaHCO_3$ and H_2O, dried, and the solvent removed *in vacuo*. The residue (foam, 750 mg) is dissolved in methanol–ether, silica gel (5 g) is added and the solvent removed *in vacuo*. This material is transferred onto a column of silica gel (20 g) in ether. The column is eluted with ether which removes the less polar impurities (40 mg). Ensuing elution with ether–methanol (20%) yields the product *XL* (700 mg, amorphous), $[\alpha]_D^{20}$ +24° (*c* 1·4). IR spectrum (chloroform): 3 615, 3 585, 1 068 (OH), 3 440, 1 713, 1 518 (CONH), 1 725, 1 187, 1 173 (ester, lactone) cm^{-1}. For $C_{33}H_{47}NO_8$ (585·7) calculated: 67·67% C, 8·09% H, 2·39% N; found: 67·45% C, 8·02% H, 2·32% N.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-L-Leucinate (*XLI*)

The compound *XL* (650 mg) is dossolved in methanol (40 ml), Pd/C (10%, 500 mg), washed with

methanol before use, is added and a stream of hydrogen is passed through the mixture with magnetic stirring. The reaction is completed after 45 min, the catalyst is filtered off, washed with methanol and the filtrate evaporated. The residue (470 mg) is dissolved in methanol, adsorbed on silica gel (5 g, washed with methanol before use until no inorganic material is eluted) and the solvent removed on a rotary evaporator. This material is then transferred to a column of silica gel (10 g, also washed with methanol before use, the methanol is then replaced with ether) and eluted with ether–methanol (5%). After removing the less polar impurities, the product (232 mg, amorphous) shows the IR spectrum (chloroform): 1 729 (OCO), 3 610, 3 580, 1 189 (hydroxyl) cm^{-1}. For $C_{25}H_{41}NO_6$ (451·6) calculated: 66·49% C, 9·15% H, 3·10% N; found: 66·11% C, 9·30% H, 2·85% N.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-(N-Benzyloxycarbonylglycinate) (*XLII*)

The isopropylidene derivative *XXXIX* (770 mg) is suspended in a methanol (24 ml)–benzene (4 ml) mixture and 0·4 ml conc. HCl and shaken occasionally. After 1·5 h the mixture is concentrated at reduced pressure and room temperature, diluted with water and taken up in benzene–ether (the product is not readily soluble). The solution is washed with water, $NaHCO_3$ and water, dried with $MgSO_4$ and evaporated. The residue is dissolved in methanol, silica gel (4 g) is added and the solvent removed on a rotatory evaporator. This material is transferred onto a silica gel column (18 g) in ether. Elution with ether separates some less polar material (20 mg) followed by the product (650 mg, amorphous), $[\alpha]_D^{20}$ +21° (*c* 2). IR spectrum (chloroform): 3 460, 1 723, 1 710, 1 521 (NHCOO), 1 723 (OCO), 3 630, 3 620, (OH), 704 ($C_6H_5CH_2$) cm^{-1}. For $C_{29}H_{39}NO_8$ (529·6) calculated: 65·77% C, 7·42% H, 2·65% N; found: 65·67% C, 7·32% H, 2·57% N.

2α,3α,17β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one 17-Glycinate (*XLIII*)

The carbobenzoxy derivative *XLII* (600 mg) is dissolved in methanol. Pd/C catalyst (10%, 500 mg) is added and the same procedure is used as in the case of *XLI*. Chromatography on 20 g of silica gel (washed with methanol as above) in ether–methanol (5%) gives *XLIII* (amorphous), $[\alpha]_D^{20}$ +34° (*c* 1·6). IR spectrum (chloroform): 3 615, 3 585, 1 070, 1 028 (hydroxyl), 1 728, 1 186, 1 170 (lactone), 3 395, 1 728, 1 606, 1 236, 1 200 ($OCOCH_2NH_2$) cm^{-1}. For $C_{21}H_{33}NO_6$ (395·5) calculated: 63·78% C, 8·41% H, 3·54% N; found: 63·52% C, 8·57% H, 3·30% N.

Elemental analyses were carried out in the Analytical Laboratory of this Institute by Mrs E. Sýkorová, V. Rusová, and E. Šípová under the direction of Dr. J. Horáček. The authors are indebted to Dr J. Smolíková for the interpretation of the IR *spectra, measured by Mrs K. Matoušková and Mrs H. Šulcová. We thank Dr J. Zajíček and Dr M. Budějínský for the measurement and interpretation of the 200 MHz* ^{1}H NMR *spectra. The 60 MHz* ^{1}H NMR *spectra were measured by Mrs J. Jelínková and M. Snopková. The mass spectra were measured by Mrs P. Loudová.*

REFERENCES

1. Kohout L., Strnad M., Kamínek M.: This Journal *51*, 447 (1986).
2. Kohout, L., Velgová H., Strnad M., Kamínek M.: This Journal *52*, (1987).
3. Kohout L., Velgová H., Strnad M.: Czech. Appl. 1745—86.
4. Černý V., Strnad M., Kamínek M.: This Journal *51*, 687 (1986).
5. Černý V., Kamínek M., Strnad M.: Czech. 242122.
6. Černý V., Strnad M., Kamínek M.: This Journal, in press.

7. Černý V., Zajíček J., Strnad M.: This Journal *52*, 215 (1987).
8. Kondo M., Mori K.: Agr. Biol. Chem. *47*, 97 (1983).
9. Robinson C. H., Gnoj O., Carlon F. E.: Tetrahedron *21*, 2509 (1956).
10. Velgová H., Synáčková M., Černý V.: This Journal *44*, 260 (1979).
11. Takatsuto S., Ikekawa N.: Tetrahedron Lett. *24*, 917 (1983).
12. Strnad M., Motyka V., Kamínek M.: *12th International Conference on Plant Growth Substances*, Heidelberg, August 1985, Abstract No. 0605.
13. Strnad M., Černý V., Kohout L., Kamínek M.: *Biological Acitivities of Some Brassinosteroids in Two Different Bean Internode Bioassays.* Symposium "Regulation of Plant Integrity" September 1985, Brno, Czechoslovakia.
14. Velgová H., Černý V., Šorm F.: This Journal *37*, 1015 (1972).

Translated by the author (V. Č.).