SYNTHESIS OF BRASSINO STEROIDS WITH A FIVE CARBON ATOM ESTER FUNCTIONALITY IN POSITION 17*

Ladislav KOHOUT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received February 14, 1989
Accepted April 3, 1989

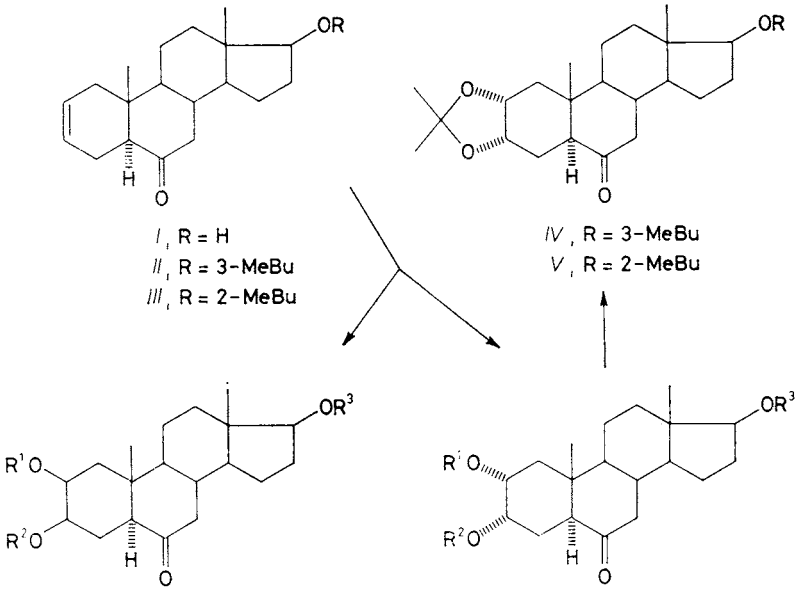
Androstane analogues of brassinolide with a five carbon atom ester functionality in position 17 have been prepared. 2α,3α-Dihydroxy-17β-(3-methylbutyryloxy)-7-oxa-B-homo-5α-androstan-6-one (*XVIII*) exhibited a surprisingly high brassinolide activity.

The natural plant hormone brassinolide ((22*R*,23*R*,24*S*)-2α,3α-22,23-tetrahydroxy-24-methyl-7-oxa-B-homo-5α-cholestan-6-one), isolated several years ago[1], exhibits significant growth-promoting activity. In the course of our structure–activity studies on brassino steroids[2-5] we have prepared brassinolide analogs bearing in position 17 an ester group derived from 2-methylbutyric and 3-methylbutyric acid.

The compounds were synthesized starting from the known[6] 17β-hydroxy-5α-androst-2-en-6-one (*I*). We first prepared the 3-methylbutyrate *II* and 2-methylbutyrate *III* by reaction of *I* with the corresponding acyl chlorides. The double bond in the compounds *II* and *III* was hydroxylated with N-methylmorpholine N-oxide in the presence of osmium tetroxide. The hydroxylation gave the respective 2α,3α-diols *X* and *XI*, along with minor amounts of the 2β,3β-diols which could be removed by crystallization. Treatment of the diol *X* with acetic anhydride for 24 hours led to a mixture of diacetate *XIV* and monoacetate *XII*. Analogously, diol *XI* afforded diacetate *XV* and monoacetate *XIII*. The structure of the monoacetates follows from an analogous partial acetylation of a 2α,3α-diol, described in the literature[4]. The monoacetates *XII* and *XIII* were converted into the diacetates by using prolonged reaction time or higher temperature (e.g. with acetic anhydride in boiling pyridine). Acetylation of mother liquors from crystallization of diols *X* and *XI* furnished 2β,3β-diacetates *VIII* and *IX*, together with the corresponding monoacetates *VI* and *VII* and 2α,3α-diacetates *XIV* and *XV*. The structure of the monoacetates also follows from an analogous partial acetylation of 2β,3β-diols, described in the literature[2,4].

* Part CCCXLVIII in the series On Steroids Part CCCXLVII: Collect. Czech. Chem. Commun. *54*, 3339 (1989).

From the diols *X* and *XI* we further prepared acetals *IV* and *V* by reaction with acetone and anhydrous copper(II) sulfate under catalysis with *p*-toluenesulfonic acid monohydrate.



I, R = H
II, R = 3-MeBu
III, R = 2-MeBu

IV, R = 3-MeBu
V, R = 2-MeBu

VI, R^1 = H ; R^2 = Ac ; R^3 = 3-MeBu
VII, R^1 = H ; R^2 = Ac ; R^3 = 2-MeBu
VIII, R^1 = R^2 = Ac ; R^3 = 3-MeBu
IX, R^1 = R^2 = Ac ; R^3 = 2-MeBu

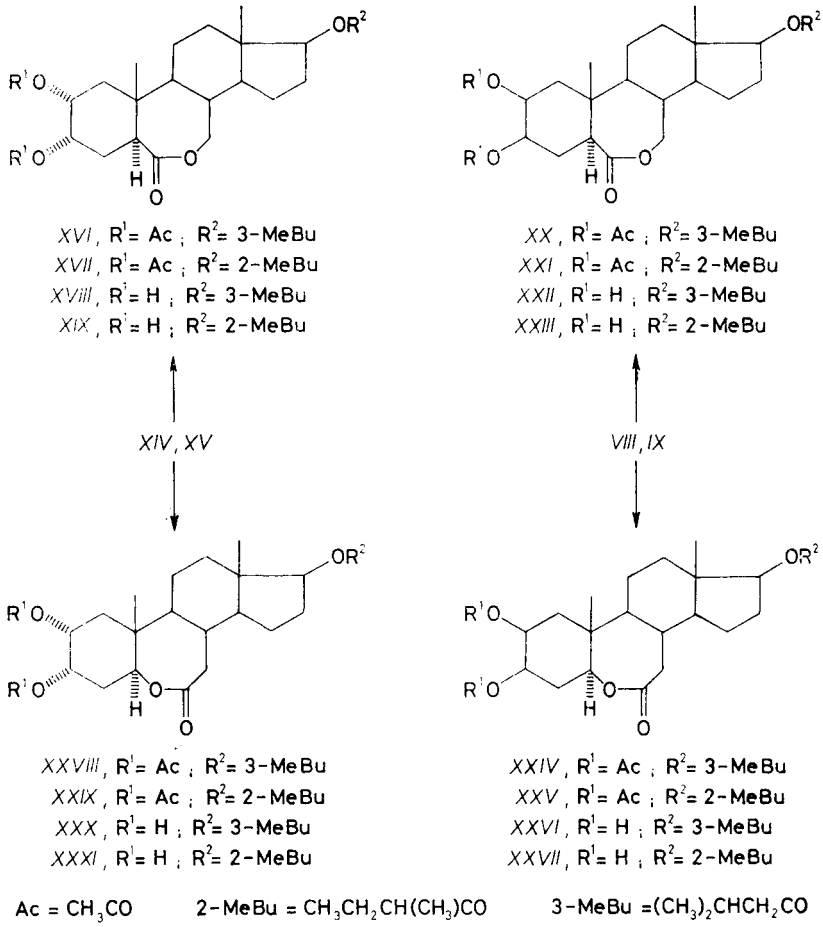
X, R^1 = R^2 = H ; R^3 = 3-MeBu
XI, R^1 = R^2 = H ; R^3 = 2-MeBu
XII, R^1 = Ac ; R^2 = H ; R^3 = 3-MeBu
XIII, R^1 = Ac ; R^2 = H ; R^3 = 2-MeBu
XIV, R^1 = R^2 = Ac ; R^3 = 3-MeBu
XV, R^1 = R^2 = Ac ; R^3 = 2-MeBu

Ac = CH_3CO 2-MeBu = $CH_3CH_2CH(CH_3)CO$ 3-MeBu = $(CH_3)_2CHCH_2CO$

All the obtained diacetates, i.e. *VIII*, *IX*, *XIV*, and *XV*, were converted into the corresponding lactones by oxidation with trifluoroperoxyacetic acid in dichloromethane. In all cases the reaction afforded a mixture of isomeric 7-oxa-B-homo-6--ketone and 6-oxa-B-homo-7-ketone, in which the first predominated. Selective hydrolysis of the acetate groups in diacetates *XX*, *XXI*, *XVI*, *XVII*, *XXIV*, *XXV*, *XXVIII*, and *XXIX* without saponification of the 17β-ester group was performed by treatment with potassium carbonate in methanol at room temperature for less than 1 hour and led to the corresponding diols *XVIII*, *XIX*, *XXII*, *XXIII*, *XXVI*, *XXVII*, *XXX*, and *XXXI*. The decision between the 7-oxa-B-homo-6-ketone or 6-oxa-B-homo-7-ketone structure was made on the basis of ^{1}H NMR spectroscopy: the spectra of 7-oxa-derivatives (*XVIII*, *XIX*, *XXII*, and *XXIII*) exhibit two 7a--proton signals at 4·03 ± 0·03 ppm whereas the 6-oxa-derivatives (*XXVI*, *XXVII*,

XXX, and *XXXI*) display only one proton signal (H-5) in the region 4·4–4·8 ppm.

These two isomeric structures were also distinguished by mass spectrometry. We compared a series of 2,3-diols of the 7-oxa series (*XVIII*, *XIX*, *XXII*, and *XXIII*) with the corresponding 6-oxa derivatives (*XXVI*, *XXVII*, *XXX*, and *XXXI*). Spectra of the former series exhibit a very abundant peak at *m/z* 377 which is absent in the



spectra of the 6-oxa compounds. On the other hand, the 6-oxa compounds have strong peaks at *m/z* 375, 349 and 305, not discernible in the spectra of 7-oxa derivatives. This behaviour can be explained by different fragmentation: the 7-oxa compounds lose H_2O and C_2H_3 under formation of a fragment of *m/z* 377, whereas the 6-oxa derivatives lose H_2O and CHO (fragment *m/z* 375), C_2H_2 (*m/z* 349) and C_2H_4O (*m/z* 305). The composition of all the ions was confirmed by high resolution experiments.

As follows from preliminary results of second internode assay[7], lactone *XVIII* exhibits high brassinolide activity.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorected. Optical rotations were measured in chloroform (unless stated otherwise), error ± 3°. Infrared spectra were recorded on a Zeiss UR20 spectrometer in tetrachloromethane (unless stated otherwise); wavenumbers in cm^{-1}. Proton NMR spectra were taken on a Tesla BS 497 (100 MHz) instrument in deuterochloroform with tetramethylsilane as internal standard (unless stated otherwise). Chemical shifts are given in ppm (δ-scale), coupling constants (J) and multiplet half-widths ($W_{1/2}$) in Hz. The symbol $W_{1/2}$ denotes the signal width in half of its height. The data were interpreted as the first-order spectra. Mass spectra were obtained with a ZAB-EG spectrometer at 70 eV. The identity of the prepared samples was checked by mixture melting points, thin-layer chromatography (TLC), IR spectra and ^{1}H NMR spectra. Preparative TLC was carried out on 200 × × 200 mm plates coated with 0·7 mm thick layer of silica gel Woelm DC. "Usual work-up of a solution" denotes washing with 5% hydrochloric acid, water, 5% aqueous potassium hydrogen carbonate, water, drying over sodium sulfate, filtering and evaporation of the solvent in vacuo to dryness. The light petroleum used was a fraction boiling at 40–62°C.

17β-(3-Methylbutyryloxy)-5α-androst-2-en-6-one (*II*)

3-Methylbutyryl chloride (1 ml) was added to a solution of 17β-hydroxy-5α-androst-2-en-6-one[6] (*I*, 0·50 g) in pyridine (5 ml). After standing at room temperature for 2 h, the mixture was poured into an ice–water mixture, extracted with ether and worked up in the usual manner. The crude product (580 mg) was purified by chromatography on silica gel (100 g) in benzene–ether (19 : 1). The obtained ester *II* (280 mg) melted at 145–147°C (ethanol), $[\alpha]_D^{20}$ +16° (*c* 1·28). IR spectrum: 3 025, 3 060, 1 675 (double bond); 1 733, 1 186 (ester); 1 713 (6-ketone). ^{1}H NMR spectrum: 0·71 s, 3 H and 0·79 s, 3 H (3 × H-18 and 3 × H-19); 0·94 d, 6 H (methyl ester, J = 6 Hz); 5·63 m, 1 H (H-17α, $W_{1/2}$ = 18 Hz); 5·61 brs, 2 H (H-2 and H-3, $W_{1/2}$ = 6 Hz). For $C_{24}H_{36}O_3$ (372·5) calculated: 77·38% C, 9·74% H; found: 73·09% C, 9·97% H.

17β-(2-Methylbutyryloxy)-5α-androst-2-en-6-one (*III*)

2-Methylbutyryl chloride (1 ml) was added to a solution of 17β-hydroxy-5α-androst-2-en-6-one[6] (*I*, 0·50 g) in pyridine (5 ml). The mixture was worked up as described in the preceding experiment, affording 340 mg of ester *III*, m.p. 137–140°C (ethanol), $[\alpha]_D^{20}$ +14° (*c* 1·26). IR spectrum: 3 060, 3 025, 1 658, 670 (double bond); 1 734, 1 185 (ester); 1 715 (6-ketone). ^{1}H NMR spectrum: 0·72 s, 3 H and 0·80 s, 3 H (3 × H-18 and 3 × H-19); 1·13 d, 3 H (CH_3–CH– of the ester, J = 6·5 Hz); 0·895 t, 3 H (CH_3–CH_2– of the ester, J = 7 Hz); 4·62 m, 1 H (H-17α, $W_{1/2}$ = = 15 Hz); 5·61 brs, 2 H (H-2 and H-3, $W_{1/2}$ = 5 Hz). For $C_{24}H_{36}O_3$ (372·5) calculated: 77·38% C, 9·74% H; found: 77·03% C, 9·74% H.

2α,3α-Isopropylidenedioxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*IV*)

Anhydrous copper(II) sulfate (393 mg) and *p*-toluenesulfonic acid monohydrate (50 mg) were added to a solution of diol *X* (393 mg) in acetone (20 ml). After stirring at room temperature overnight, the mixture was poured into a saturated aqueous solution of potassium carbonate and the product was extracted with ether. Purification by chromatography on a column of silica

gel (100 g) in light petroleum–ether (3 : 1) gave 350 mg of compound *IV*, m.p. 182–184°C (subl.). IR spectrum: 1 733, 1 188 (ester); 1 716 (6-ketone); 1 381, 1 369, 1 247, 1 220, 1 061 and 1 022 (isopropylidenedioxy group). ^{1}H NMR spectrum: 0·685 s, 3 H (3 × H-19); 0·78 s, 3 H (3 × × H-18); 0·95 d, 6 H (methyl ester, J = 6·5 Hz); 1·34 s, 3 H and 1·50 s, 3 H (isopropylidenedioxy group); 3·86 and 4·38 m, 2 H (H-2β and H-3β); 4·62 m, 1 H (H-17α, $W_{1/2}$ = 17·5 Hz). For $C_{27}H_{42}O_5$ (446·6) calculated: 72·61% C, 9·48% H, found: 72·51% C, 8·95% H.

2α,3α-Isopropylidenedioxy-17β-(2-methylbutyryloxy)-5α-androstan-6-one (*V*)

Anhydrous copper(II) sulfate (290 mg) and *p*-toluenesulfonic acid monohydrate (50 mg) were added to a solution of diol *XI* (290 mg) in acetone (15 ml). The mixture was processed as described in the preceding experiment; the crude product (240 mg) was crystallized from ethanol, affording 260 mg of the title compound *V*, m.p. 200–202°C (subl.). IR spectrum: 1 731, 1 184 (ester); 1 716 (6-ketone); 1 380, 1 367, 1 295, 1 228, 1 060 and 1 020 (isopropylidenedioxy group). ^{1}H NMR spectrum: 0·78 s, 3 H (3 × H-18); 0·68 s, 3 H (3 × H-19); 1·12 d, 3 H (CH_3–CH– in the ester, J = 7 Hz); 0·89 t, 3 H (CH_3–CH_2– in the ester, J = 7 Hz); 1·33 s, 3 H and 1·49 s, 3 H (methyl groups of the isopropylidenedioxy group); 3·86–4·38 m, 2 H (H-2α and H-3α); 4·63 m, 1 H (H-17α, $W_{1/2}$ = 18 Hz). For $C_{27}H_{42}O_5$ (446·6) calculated: 71·61% Ct 9·48% H; found: 72·36% C, 9·67% H.

2β,3β-Diacetoxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*VIII*)

The mother liquors (0·58 g) from crystallization of the diol *X* were dissolved in pyridine (10 ml) and acetic anhydride (2 ml) and set aside at room temperature for 4 days. The mixture was poured into an ice–water mixture, the product was taken up in ether and worked up in the usual manner. Chromatography on a column of silica gel (550 g) in light petroleum–ether (3 : 1) afforded (along with diacetate *XIV*) 80 mg of diacetate *VIII*, m.p. 194–196°C (subl.), $[\alpha]_D^{20}$ +16° (*c* 0·5). IR spectrum (KBr): 1 740, 1 244, 1 193 (esters); 1 706 (6-ketone). ^{1}H NMR spectrum: 0·78 s, 3 H (3 × H-18); 0·925 s, 3 H (3 × H-19); 0·935 d, 6 H (methyl groups in the 17β-ester, J = 6 Hz); 1·98 s, 3 H and 2·05 s, 3 H (2 × $OOCCH_3$); 4·23 m, 1 H (H-3α, $W_{1/2}$ = 16 Hz); 4·65 mt 1 H (H-17α, $W_{1/2}$ = 18 Hz); 5·23 m, 1 H (H-2α, $W_{1/2}$ = 13 Hz). For $C_{28}H_{42}O_7$ (490·6) calculated: 68·54% C, 8·63% H; found: 68·30% C, 8·89% H.

2β,3β-Diacetoxy-17β-(2-methylbutyryloxy)-5α-androstan-6-one (*IX*)

The mother liquors (242 mg) from the crystallization of diol *XI* were acetylated with acetic anhydride (1 ml) in pyridine (5 ml) using the procedure described in the preceding experiment. Chromatography on a column of silica gel (200 g) in light petroleum–ether (2 : 1) afforded (along with 123 mg of diacetate *XV*) the diacetate *IX* (67 mg), m.p. 202–205°C (subl.). IR spectrum (KBr): 1 736, 1 260, 1 248, 1 193 (esters); 1 702 (6-ketone). ^{1}H NMR spectrum: 0·78 s, 3 H (3 × H-18); 0·92 s, 3 H (3 × H-19); 0·875 t, 3 H (CH_3CH_2– 17β-ester, J = 7 Hz); 1·11 d, 3 H (CH_3CH– 17β-ester, J = 7 Hz); 1·97 s, 3 H and 2·05 s, 3 H (2 × $OOCCH_3$); 4·22 m, 1 H (H-3α, $W_{1/2}$ = 10 Hz); 4·63 t, 1 H (H-17α, J = 7·5 Hz); 5·27 m, 1 H (H-2α, $W_{1/2}$ = 18 Hz). For $C_{28}H_{42}O_7$ (490·6) calculated: 68·55% C, 8·63% H; found: 68·39% C, 8·89% H.

2α,3α-Dihydroxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*X*)

A) From 17β-(3-methylbutyryloxy)-5α-androst-2-en-6-one (*II*). A solution of olefin *II* (256 mg) in acetone (15 ml) was mixed with a solution of osmium tetroxide (12·2 mg) in tert-

-butyl alcohol (0·122 ml), tert-butyl alcohol (0·5 ml), N-methylmorpholine N-oxide (256 mg) and water (0·62 ml). After standing for 48 h under nitrogen, 10% solution of sodium sulfite (1·3 ml) was added and the mixture was stirred for 40 min at room temperature. The product was extracted with ether and processed in the usual manner to give 318 mg of material which was purified by preparative TLC on silica gel (14 plates) in chloroform–ether (1 : 1). The obtained product (183 mg) was crystallized from ethanol, yield 109 mg of *X*, m.p. 179–182°C, $[\alpha]_D^{20}$ −13° (*c* 1·2). IR spectrum: 3 625 sh, 3 430 (hydroxyls); 1 727, 1 193 (ester); 1 711 sh (6-ketone). ^{1}H NMR spectrum: 0·76 s, 3 H and 0·78 s, 3 H (3 × H-18 and 3 × H-19); 0·95 bd, 6 H (protons of methyl esters); 3·64 m, 1 H (H-2β, $W_{1/2}$ = 22); 4·00 m, 1 H (H-3β, $W_{1/2}$ = 9 Hz); 4·63 t, 1 H (H-17α, J = 7·5 Hz). For $C_{24}H_{38}O_5$ (406·5) calculated: 70·90% C, 9·42% H; found: 71·17% C, 9·53% H.

B) From 2α,3α-diacetoxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*XIV*). Potassium carbonate (47 mg) in water (0·7 ml) was added to a solution of diacetate *XIV* (95 mg) in methanol (7 ml). After standing at room temperature for 30 min, the product was extracted with water, the extract was dried and the solvent evaporated. The residue (68 mg) was crystallized from aqueous ethanol to afford 41 mg of diol *X*, m.p. 172–178°C, $[\alpha]_D^{20}$ −8° (*c* 1·1).

C) From 2α,3α-isopropylidenedioxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*V*). A solution of compound *V* (100 mg) in methanol (10 ml) was mixed with 37% hydrochloric acid (0·2 ml). After standing at room temperature for 30 min, the solvent was evaporated in vacuo at 40°C. The residue was coevaporated with a mixture of benzene (10 ml) and ethanol (10 ml) and the remaining material (85 mg) was crystallized from ethanol to give 45 mg of diol *X*, m.p. 176–180°C, $[\alpha]_D^{20}$ −11° (*c* 1·1).

2α,3α-Dihydroxy-17β-(2-methylbutyryloxy)-5α-androstan-6-one (*XI*)

A) From 17β-(2-methylbutyryloxy)-5α-androst-2-en-6-one (*III*). A mixture of olefin *III* (305 mg) in acetone (18 ml), osmium tetroxide (0·15 g) in tert-butyl alcohol (0·15 ml), tert-butyl alcohol (0·6 ml), N-methylmorpholine N-oxide (305 mg) and water (0·75 ml) was allowed to stand for 2 days under nitrogen. After addition of a 10% sodium sulfite solution (1·5 ml) the mixture was stirred for 30 min. The product was taken up in ether and worked up in the usual manner. TLC on silica gel (19 plates) in chloroform–ether (1 : 1) afforded 262 mg of product which was crystallized from ethanol to furnish 234 mg of diol *XI*, m.p. 178–182°C, $[\alpha]_D^{20}$ −12° (*c* 1·3). IR spectrum (chloroform): 3 620, 3 586, 3 490, 1 044 (hydroxyl groups); 1 720, 1 195 (ester); 1 711 sh (6-ketone). ^{1}H NMR spectrum: 0·77 s, 3 H and 0·80 s, 3 H (3 × H-18 and 3 × H-19); 0·90 t, 3 H (protons of the CH_3CH_2–group in the ester); 1·11 d, 3 H (protons of the CH_3CH–group in the ester, J = 7 Hz); 3·68 m, 1 H (H-2β, $W_{1/2}$ = 24 Hz); 4·00 m, 1 H (H-3β, $W_{1/2}$ = 8 Hz); 4·64 t, 1 H (H-17α, J = 7·5 Hz). For $C_{24}H_{38}O_5$ (406·5) calculated: 70·90% C, 9·42% H; found: 70·92% C, 9·40% H.

B) From 2α,3α-diacetoxy-17β-(2-methylbutyryloxy)-5α-androstan-6-one (*XV*). A solution of diacetate *XV* (130 mg) and potassium carbonate (70 mg) in a mixture of methanol (20 ml) and water (2 ml) was allowed to stand for 65 min at room temperature. The reaction mixture was poured into water and the product was taken up in chloroform. The chloroform extract was washed with water, dried over sodium sulfate, and the solvent was evaporated. The residue (65 mg) was crystallized from ethanol; yield 21 mg of crystals, m.p. 182–184°C, $[\alpha]_D^{20}$ −9° (*c* 1·4).

2α,3α-Diacetoxy-17β-(3-methylbutyryloxy)-5α-androstan-6-one (*XIV*)

A mixture of diol *X* (2·3 g), pyridine (12 ml) and acetic anhydride (4 ml) was set aside for 2 days at room temperature. The reaction mixture was poured into an ice–water mixture, the product

was extracted with ether and worked up as usual. Chromatography on a column of silica gel (200 g) in light petroleum–ether (2 : 1) afforded the desired product *XIV* as the main lipophilic fraction (1·92 g), together with a minor polar product (37 mg, monoacetate *XII*). This minor product was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (0·5 ml), allowed to stand for 4 days at room temperature and then extracted with ether. The extract was worked up as usual and afforded a further portion (40 mg) of product, identical with the main lipophilic fraction (vide supra). Crystallization from ethanol furnished 1·38 g of diacetate *XIV*, m.p. 192–193°C (subl.), $[\alpha]_D^{20}$ 0° (*c* 0·75). IR spectrum: 1 747, 1 259 (acetate); 1 724, 1 190 (17β-ester); 1 711 sh (6-ketone). ^{1}H NMR spectrum: 0·80 s, 3 H (3 × H-18); 0·84 s, 3 H (3 × H-19); 0·95 d, 6 H (methyl protons of the 17β-ester, J = 6 Hz); 1·98 s, 3 H and 2·075 s, 3 H (2 × $OOCCH_3$); 4·48–5·20 m, 2 H (H-2β and H-17α); 5·33 m, 1 H (H-3β, $W_{1/2}$ = 9 Hz). For $C_{28}H_{42}O_7$ (490·6) calculated: 68·54% C, 8·63% H; found: 68·70% C, 9·11% H.

2α,3α-Diacetoxy-17β-(2-methylbutyryloxy)-5α-androstan-6-one (*XV*)

A solution of diol *XI* (2·12 g) in pyridine (10 ml) was mixed with acetic anhydride (4 ml) and the mixture was set aside at room temperature overnight. After pouring into water, the product was taken up in ether and the extract was worked up in the usual manner. The residue was chromatographed on a column of silica gel (200 g) in light petroleum–ether (2 : 1), affording 1·89 g of *XV* as the main lipophilic product and 31 mg of monoacetate *XIII* as the minor (polar) product. The latter was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (0·5 ml) and allowed to react at room temperature for 4 days. The usual work-up afforded further 33 mg of a product identical with the main, lipophilic, product. Crystallization from ethanol furnished 16·5 mg of diacetate *XV*, m.p. 195–296°C (subl.). IR spectrum (KBr): 1 738, 1 260, 1 192 (esters); 1 704 (6-ketone). ^{1}H NMR spectrum: 0·80 s, 3 H and 0·83 s, 3 H (3 × H-18 and 3 × H-19), 0·90 t, 3 H (CH_3CH_2-protons of 17β-ester, J = 6·5 Hz); 1·12 d, 3 H (CH_3CH-protons of 17β-ester, J = 6·5 Hz); 1·97 s, 3 H and 2·06 s, 3 H (2 × $OOCCH_3$); 4·38–5·20 m, 2 H (H-2β and H-17α); 5·37 mt 1 H (H-3β, $W_{1/2}$ = 8 Hz). For $C_{28}H_{42}O_7$ (490·6) calculated: 68·55% C, 8·63% H; found: 68·49% Ct 8·92% H.

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

To a solution of ketone *XIV* (1·1 g) in dichloromethane (12 ml) was added a solution of trifluoroperoxyacetic acid in dichloromethane (prepared from 3·54 g of trifluoroacetic anhydride and 0·36 ml of 50% hydrogen peroxide in 30 ml of dichloromethane). After standing at room temperature for 8 h, the reaction mixture was poured into water, the product was extracted with chloroform and the extract was worked up in the usual manner. The product which contained two compounds, was chromatographed on a column of silica gel (250 g) in light petroleum–ether (2 : 1). Fractions, containing the lipophilic product, afforded 1·55 g of lactone *XVI* as an oil. IR spectrum: 1 748, 1 249 (acetates); 1 748, 1 187, 1 172 (17β-ester); 1 731 sh, 1 297, 1 022 (lactone). ^{1}H NMR spectrum: 0·82 s, 3 H (3 × H-18); 1·00 s, 3 H (3 × H-19); 0·95 d, 6 H (methyl groups of the 17β-ester, J = 6 Hz); 1·98 s, 3 H and 2·10 s, 3 H (2 × $OOCCH_3$); 2·99 m, 1 H (H-5α, $W_{1/2}$ = 29 Hz); 4·07 d, 2 H (2 × H-8a, J = 5 Hz); 4·41–5·06 m, 2 H (H-2β and H-17α); 5·33 brs, 1 H (H-3β, $W_{1/2}$ = 8·5 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·70% C, 8·40% H.

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

To a solution of ketone *XV* (880 mg) in dichloromethane (18 ml) was added a solution of trifluoroperoxyacetic acid in dichloromethane (42 ml) (prepared from 5·3 g of trifluoroacetic an-

hydride and 0·53 ml of 50% hydrogen peroxide). After standing for 66 h at room temperature, the reaction mixture was poured into 10% potassium hydrogen carbonate solution. The product was taken up in chloroform, the chloroform extract was washed with water, dried over sodium sulfate and the solvent was evaporated. The residue (950 mg) was chromatographed on a silica gel column (250 g) in light petroleum–ether (7 : 3) to give 680 mg of the lipophilic product which on crystallization from aqueous ethanol afforded 541 mg of lactone *XVII*, m.p. 160–162°C (change of modification at 120–130°C). IR spectrum] 1 749, 1 250 (acetates); 1 730 sh, 1 186, 1 155, 1 083 (lactone and 17β-ester). ^{1}H NMR spectrum: 0·82 s, 3 H (3 × H-18); 0·98 s, 3 H (3 × H-19); 0·90 t, 3 H (CH_3CH_2-protons of 17β-ester, J = 6·5 Hz); 1·12 d, 3 H (CH_3CH-protons of 17β-ester, J = 6·5 Hz); 1·98 s, 3 H and 2·10 s, 3 H (2 × $OOCCH_3$); 4·08 d, 2 H (2 × H-7a, J = 5 Hz); 4·45–5·12 mt 2 H (H-2β and H-17α); 5·35 m, 1 H (H-3β, $W_{1/2}$ = 7 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·51% C, 8·36% H.

2α,3α-Dihydroxy-17β-(3-methylbutyryloxy)-7-oxa-B-homo-5α-androstan-6-one (*XVIII*)

A solution of potassium carbonate (127 mg) in water (1·3 ml) was added to a solution of diacetate *XVI* (219·5 mg) in methanol (14 ml). After standing at room temperature for 30 min, the mixture was partitioned between water and chloroform, the organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure. The residue (295 mg) was chromatographed on silica gel (200 g) in chloroform–ether (1 : 1) to give 141 mg of noncrystalline diol *XVIII*. IR spectrum: 3 445 (hydroxyl groups); 1 837, 1 189 (esters); 1 728, 1 712 and 1 297 (lactone). Mass spectrum, m/z: 422 (M^+), 404, 377, 335. ^{1}H NMR spectrum: 0·82 s, 3 H (3 × × H-18); 0·92 s, 3 H (3 × H-19); 0·95 d, 6 H (methyl ester, J = 6 Hz); 3·42–4·18 m, 2 H (H-2β and H-3β); 4·05 bd, 2 H (2 × H-7a, J = 5 Hz); 4·61 m, 1 H (H-17α, $W_{1/2}$ = 29 Hz). For $C_{24}H_{38}O_6$ (422·6) calculated: 68·22% C, 9·06% H; found: 67·99% C, 9·01% H.

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

A solution of potassium carbonate (203 mg) in water (2·2 ml) was added to a solution of diacetate *XVII* (360 mg) in methanol (22 ml). After standing at room temperature for 40 min, the mixture was poured into water and extracted with chloroform. The chloroform solution was separated, dried over anhydrous sodium sulfate and the solvent was evaporated under diminished pressure. The residue (380 mg) was chromatographed on a column of silica gel (50 g) in chloroform–ether (1 : 1); yield 272 mg of non-crystalline *XIX*. IR spectrum (chloroform): 3 615, 3 580 (hydroxyl groups); 1 723, 1 712 sh, 1 291, 1 161 (ester and lactone). Mass spectrum, m/z: 422 (M^+), 404, 377, 335. ^{1}H NMR spectrum: 0·82 s, 3 H (3 × H-18); 0·92 s, 3 H (3 × H-19); 0·89 t, 3 H (CH_3CH_2-protons of ester, J = 6·5 Hz); 1·12 d, 3 H (CH_3CH-protons of ester, J = 6·5 Hz); 3·44–4·21 m, 2 H (H-2β and H-3β); 4·06 d, 2 H (2 × H-7a, J = 5 Hz); 4·64 m, 1 H (H-17α, $W_{1/2}$ = 7 Hz). For $C_{24}H_{38}O_6$ (422·6) calculated: 68·22% C, 9·06% H; found: 67·85% C, 8·65% H.

2β,3β-Diacetoxy-17β-(3-methylbutyryloxy)-7-oxa-B-homo-5α-androstan-6-one (*XX*)

To a solution of ketone *VIII* (449 mg) in dichloromethane (5 ml) was added a solution of trifluoroperoxyacetic acid in dichloromethane (12·5 ml), prepared from 1·48 g of trifluoroacetic anhydride and 0·15 ml of 50% hydrogen peroxide. After standing at room temperature for 2 h, the reaction mixture was poured into water and extracted with chloroform. The chloroform solution was washed with 10% potassium hydrogen carbonate and water, dried over anhydrous sodium sulfate and the solvent was evaporated. The residue (365 mg) was subjected to preparative

TLC on silica gel (16 plates) in light petroleum–ether (2 : 3). Zones, containing the lipophilic product, afforded 256 mg of lactone *XX*, m.p. 249–250°C (subl., methanol). IR spectrum (chloroform): 1 735, 1 259 (acetates); 1 835, 1 298, 1 171 (17β-ester); 1 835, 1 135, 1 018 (lactone). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·94 d, 6 H (methyl groups of 17β-ester, J = 6 Hz); 1·04 s, 3 H (3 × H-19); 2·00 s and 2·07 s, 2 × 3 H (2 × $OOCCH_3$); 4·04 d, 2 H (2 × H-8a, J = 5 Hz); 4·43–5·03 m, 2 H (H-3α and H-17α); 5·21 m, 1 H (H-2α, $W_{1/2}$ = 8·5 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·20% C, 8·09% H.

2β,3β-Diacetoxy-17β-(2-methylbutyryloxy)-7-oxa-B-homo-5α-androstan-6-one (*XXI*)

Ketone *IX* (314 mg) in dichloromethane (6·3 ml) was treated at room temperature with trifluoroperoxyacetic acid, prepared from trifluoroacetic anhydride (1·88 g) and hydrogen peroxide (50%, 0·19 ml) in dichloromethane (15 ml). After standing for 6 h, the reaction mixture was poured into 10% potassium hydrogen carbonate solution and the product was taken up in chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and the solvent was evaporated under diminished pressure. The residue (300 mg) consisted of two compounds of similar R_F values (TLC). The mixture was separated on a column of silica gel (240 g) using gradient elution with benzene–ether (from 9 : 1 to 4 : 1). The more lipophilic fraction afforded 231 mg of lactone *XXI*, m.p. 276–278°C (change of modification at 220–250°C, subl.) (ethanol). IR spectrum: 1 739, 1 258 (acetoxy groups); 1 728 sh, 1 717 sh, 1 196, 1 158 (17β-ester and lactone). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·88 t, 3 H (CH_3CH_2-protons in 17β-ester, J = 6·5 Hz); 1·03 s, 3 H (3 × H-19); 1·11 d, 3 H (CH_3CH-protons in 17β-ester, J = 6·5 Hz); 1·99 s, 3 H and 2·06 s, 3 H (2 × $OOCCH_3$); 4·04 d, 2 H (2 × H-7a, J = 5·5 Hz); 4·42–5·03 m, 2 H (H-3α and H-17α); 5·23 m, 1 H (H-2α, $W_{1/2}$ = 7 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·31% C, 8·32% H.

2β,3β-Dihydroxy-17β-(3-methylbutyryloxy)-7-oxa-B-homo-5α-androstan-6-one (*XXII*)

A solution of potassium carbonate (70 mg) in water (0·8 ml) was added to a solution of diacetate *XX* (110 mg) in methanol (7·6 ml). After standing at room temperature for 40 min, the mixture was poured into water and the product was extracted with chloroform. The chloroform extract was washed with water, dried and the solvent was evaporated under diminished pressure. The residue (96 mg) was crystallized from methanol to give 64 mg of diol *XXII*, m.p. 230–232°C (subl.). IR spectrum (chloroform): 3 620, 3 580 (hyroxyl groups); 1 728, 1 297 (ester); 1 712 sh, 1 298 (lactone). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·93 d, 6 H (methyl groups in 17β-ester, J = 6·5 Hz); 0·98 s, 3 H (3 × H-19); 3·44–4·20 m, 2 H (H-2α and H-3α); 4·01 bd, 2 H (2 × H-8a, J = 5 Hz); 4·58 m, 1 H (H-17α, $W_{1/2}$ = 18 Hz). For $C_{24}H_{38}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 68·26% C, 9·09% H.

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

A solution of potassium carbonate (74 mg) in water (0·7 ml) was added to a solution of diacetate *XXI* (120 mg) in methanol (22 ml). After standing at room temperature for 30 min, the reaction mixture was poured into water, the product extracted with chloroform, the chloroform extract washed with water, dried and the solvent distilled off under diminished pressure. Crystallization of the residue (95 mg) from ethanol afforded 59 mg of diol *XXIII*, m.p. 212–214°C (subl.). IR spectrum (chloroform): 3 620, 3 570 (hydroxyl groups); 1 727, 1 195 (ester); 1 712 sh, 1 161, 1 070 (lactone). Mass spectrum, m/z: 422 (M^+), 404, 377, 339, 322. ^{1}H NMR spectrum: 0·82 s, 3 H (3 × H-18); 0·88 t, 3 H (CH_3CH_2-group of the ester, J = 6·5 Hz); 1·02 s, 3 H (3 × H-19):

1·12 d, 3 H (CH_3CH-group of the ester, J = 6·5 Hz); 3·47—4·19 m, 2 H (H-2α and H-3α); 4·03 d, 2 H (2 × H-7a, J = 5 Hz); 4·59 m, 1 H (H-17α, $W_{1/2}$ = 15·5 Hz). For $C_{24}H_{38}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 67·62% C, 8·89% H.

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

Polar product-containing zones from the preparative TLC in the preparation of lactone *XX* afforded 55 mg of lactone *XXIV*, m.p. 242—244°C (subl., methanol). IR spectrum (chloroform): 1 837, 1 257 (acetoxy groups); 1 737, 1 300, 1 080 (lactone); 1 737, 1 194 (17β-ester). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·95 d, 6 H (two methyl groups of 17β-ester, J = 6·5 Hz); 1·05 s, 3 H (3 × H-19); 2·02 s, 3 H and 2·09 s, 3 H (2 × $OOCCH_3$); 2·34—2·56 m, 2 H (2 × × H-7a); 4·06—4·98 m, 3 H (H-2α, H-17α and H-5α); 5·20 m, 1 H (H-3α, $W_{1/2}$ = 12 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·20% C, 8·09% H.

2β,3β-Diacetoxy-17β-(2-methylbutyryloxy)-6-oxa-B-homo-5α-androstan-7-one (*XXV*)

Polar product-containing fractions from the chromatography in the preparation of lactone *XXI* afforded 48 mg of lactone *XXV*, m.p. 263—264°C (change of modification at 210—250°C, subl.) (ethanol). IR spectrum: 1 743, 1 248 (acetoxy groups); 1 729 sh, 1 712 sh, 1 189, 1 156, 1 080 (lactone, 17β-ester). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·89 t, 3 H (CH_3CH_2-group of the 17β-ester, J = 7 Hz); 1·03 s, 3 H (3 × H-19); 1·11 d, 3 H (CH_3CH-group of the 17β-ester, J = 7 Hz); 2·02 s, 3 H and 2·09 s, 3 H (2 × $OOCCH_3$); 2·36—2·59 m, 2 H (2 × H-7a); 4·20 to 5·00 m, 3 H (H-3α, H-5α and H-17α); 5·23 m, 1 H (H-2α). For $C_{48}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·24% C, 8·23% H.

2β,3β-Dihydroxy-17β-(3-methylbutyryloxy)-6-oxa-B-homo-5α-androstan-7-one (*XXVI*)

A solution of potassium carbonate (34·1 mg) in water (0·34 ml) was added to a solution of diacetate *XXIV* (65 mg) in methanol (3·4 ml). After standing at room temperature for 50 min, the reaction mixture was poured into cold water (150 ml), set aside for 10 min and the precipitated product (17 mg) was filtered. The filtrate was extracted with chloroform, the organic layer was dried over sodium sulfate and the solvent was evaporated to give another portion (16 mg) of the product. The combined portions were crystallized from aqueous methanol yielding 22 mg of diol *XXVI*, m.p. 270—273°C (subl.). IR spectrum (chloroform): 3 620, 3 570 (hydroxyl groups); 1 726, 1 300, 1 229, 1 298 (ester and lactone). Mass spectrum, m/z: 422 (M^+), 404, 375, 339, 338, 305. ^{1}H NMR spectrum: 0·78 s, 3 H (3 × H-18); 0·93 d, 6 H (methyl groups of 17β-ester, J = 6 Hz); 3·42—4·87 m, 4 H (H-2α, H-3α, H-5α, H-17α). For $C_{24}H_{38}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 68·09% C, 9·09% H.

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

A solution of potassium carbonate (20·7 mg) in water (0·23 ml) was added to a solution of diacetate *XXV* (37 mg) in methanol (6·8 ml). After stirring at room temperature for 55 min, the reaction mixture was extracted with chloroform, the chloroform solution was washed with water, dried over anhydrous sodium sulfate and the solvent was evaporated. The residue (30 mg) was crystallized from ethanol, m.p. 281—283°C (subl., decomp., change of modification at 220—250°C). IR spectrum (KBr): 3 390, 3 250 (hydroxyl groups); 1 724, 1 191 (17β-ester); 1 697, 1 159 (lactone). ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·89 t, 3 H (CH_3CH_2-group of the ester, J = 6·5 Hz); 1·10 s, 3 H (3 × H-19); 1·10 d, 3 H (CH_3CH-group of the ester,

J = 6·5 Hz); 3·31—4·78 mt 4 H (H-2α, H-3α, H-5α, H-17α). For $C_{24}H_{38}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 68·11% C, 9·11% H.

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

Polar product-containing fractions from the chromatography in the preparation of compound *XVI* afforded 93 mg of lactone *XXVIII*, m.p. 293—296°C (subl., ethanol). IR spectrum (chloroform): 1 740, 1 254 (acetoxy groups); 1 729, 1 196 (17β-ester); 1 714 sh, 1 196, 1 098 (lactone). ^{1}H NMR spectrum: 0·82 s, 3 H (3 × H-18); 0·95 d, 6 H (two methyl groups of 17β-ester, J = = 6 Hz); 1·02 s, 3 H (3 × H-19); 1·98 s, 3 H and 2·12 s, 3 H (2 × $OOCCH_3$); 4·29—5·12 m, 3 H (H-3β, H-6α, H-17α); 5·36 m, 1 H (H-3β, $W_{1/2}$ = 11 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·38% C, 8·36% H; found: 66·71% C, 8·32% H.

2α,3α-Diacetoxy-17β-(2-methylbutyryloxy)-6-oxa-B-homo-5α-androstan-7-one (*XXIX*)

Polar product-containing fractions from the chromatography in the preparation of compound *XVII* afforded 83 mg of lactone *XXIX*, m.p. 297—299°C (subl., aqueous ethanol). IR spectrum: 1 748, 1 245 (acetoxy groups); 1 748, 1 186, 1 157 (17β-ester and lactone). ^{1}H NMR spectrum: 0·83 s, 3 H (3 × H-18); 0·90 t, 3 H (CH_3CH_2-group of 17β-ester, J = 6 Hz); 1·02 s, 3 H (3 × × H-19); 1·15 d, 3 H (CH_3CH-group of 17β-ester, J = 6 Hz); 2·00 s, 3 H and 2·12 s, 3 H (2 × $OOCCH_3$); 2·40—2·62 m, 2 H (2 × H-7a); 4·29—5·26 m, 3 H (H-2β, H-5α and H-17α); 5·42 m, 1 H (H-3β, $W_{1/2}$ = 6 Hz). For $C_{28}H_{42}O_8$ (506·6) calculated: 66·39% C, 8·36% H; found: 66·49% C, 8·38% H.

2α,3α-Dihydroxy-17β-(3-methylbutyryloxy)-6-oxa-B-homo-5α-androstan-7-one (*XXX*)

A solution of potassium carbonate (28·5 mg) in water (0·3 ml) was added to a solution of diacetate *XXVIII* (51 mg) in methanol (3·1 ml). After stirring at room temperature for 30 min, the reaction mixture was poured into water and the product was taken up in chloroform. The chloroform extract was washed with water, dried over sodium sulfate and the solvent was evaporated under diminished pressure to give 41 mg of diol *XXX*, m.p. 198—202°C (subl., ethanol, change of modification at 185°C). IR spectrum (chloroform): 3 615, 3 580 (hydroxyl groups); 1 726, 1 195 (17β-ester); 1 712 sh, 1 298, 1 280 (lactone). Mass spectrum, m/z: 422 (M^+), 404, 375, 349, 305. ^{1}H NMR spectrum: 0·81 s, 3 H (3 × H-18); 0·94 s, 3 H (3 × H-19); 0·95 d, 6 H (methyl groups of 17β-ester, J = 6 Hz); 3·48—4·16 m, 2 H (H-2β and H-3β); 4·39—4·80 m, 2 H (H-5α and H-17α). For $C_{24}H_{38}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 67·70% C, 8·89% H.

2α,3α-Dihydroxy-17β-(2-methylbutyryloxy)-6-oxa-B-homo-5α-androstan-7-one (*XXXI*)

A mixture of diacetate *XXIX* (282 mg), potassium carbonate (158 mg), methanol (51·6 ml) and water (1·7 ml) was stirred at room temperature for 50 min. Analogous work-up as in the preceding experiment afforded 201 mg of material which on crystallization from aqueous ethanol gave 111 mg of diol *XXXI*, m.p. 196—197°C. IR spectrum (chloroform): 3 615, 3 570 (hydroxyl groups); 1 726, 1 198 (17β-ester); 1 712, 1 158, 1 136 (lactone). Mass spectrum, m/z: 422 (M^+), 404, 375, 349, 305. ^{1}H NMR spectrum: 0·81 s, 3 H (H-18); 0·89 t, 3 H (CH_3CH_2-group of ester, J = 7 Hz); 0·94 s, 3 H (3 × H-19); 1·12 d, 3 H (CH_3CH-group of ester, J = 7 Hz); 2·39 to 2·57 m, 2 H (2 × H-7a); 3·55—4·15 m, 2 H (H-2β and H-3β); 4·44—4·84 m, 2 H (H-5α and H-17α). For $C_{24}H_{28}O_6$ (422·5) calculated: 68·22% C, 9·06% H; found: 67·79% C, 8·92% H.

Elemental analyses were carried out in the Analytical Laboratory of this Institute by Mrs E. Sýkorová, Mrs E. Šípová and Mr V. Štěrba under the direction of Dr V. Pechanec. The author is indebted to Dr J. Smolíková for the interpretation of IR *spectra measured by Mrs K. Matoušková. Mass spectra were taken by Dr J. Kohoutová and Mrs P. Loudová from this Institute. The* ^{1}H NMR *spectra were measured by Mrs J. Jelínková and Mrs M. Snopková. A preliminary bioassay of compounds prepared in this paper was made by Dr M. Strnad of the Botanical Institute, Czechoslovak Academy of Sciences, Olomouc. The technical assistance of Mrs J. Mašková is gratefully acknowledged.*

REFERRNCES

1. Grove N. D., Spencer G. F., Rohwedder W. K., Mandava N., Worley J. F., Warthen J. D. jr, Steffens G. F., Flippen-Anderson J. L., Cook J. C. jr: Nature *281*, 216 (1979).
2. Kohout L., Strnad M.: Collect. Czech. Chem. Commun. *51*, 447 (1986).
3. Kohout L., Velgová H., Strnad M., Kamínek M.: Collect. Czech. Chem. Commun. *52*, 476 (1987).
4. Kohout L., Černý V., Strnad M.: Collect. Czech. Chem. Commun. *52*, 1026 (1987).
5. Kohout L., Strnad M.: Collect. Czech. Chem. Commun. *54*, 206 (1988).
6. Lábler L., Sláma K., Šorm F.: Collect. Czech. Chem. Commun. *33*, 2226 (1968).
7. Mitchell J. W., Livingstone G. A. in: *Methods of Studying Plant Hormones and Growth Regulating Substances*, p. 26. Agriculture Handbook No. 336, U.S. Government Printing Office, Washington D.C. 1968.

Translated by M. Tichý.