BRASSINO STEROIDS WITH ANDROSTANE AND PREGNANE SKELETON*

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Received March 7th, 1986

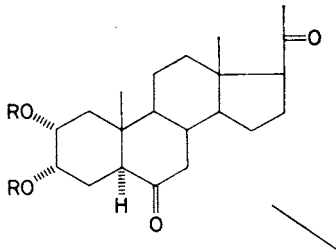
New brassino steroids *XII*, *XXI*, and *XXIII* were synthesized. These compounds, together with some intermediates of their synthesis, were tested for brassinolide activity. In the bean second internode bioassay, the androstane intermediate *V* exhibited a surprisingly high activity whereas the other tested compounds were substantially less active.

Within the framework of our investigation[1] on accessibility of brassinolide analogues we studied the applicability of pregnane derivatives to the preparation of pregnane and androstane analogues. Studies of Kondo and Mori[2], who also prepared the androstane derivatives from pregnane precursors, prompted us to describe our experiments. According to our experience, the course of the reactions employed is not always so unequivocal as described by the Japanese authors.

In their synthesis of the androstane analogue *VII*, Kondo and Mori[2] started from 5α-pregn-2-ene-6,20-dione (*I*). On hydroxylation of *I* they obtained the 2α,3α-diol *II* as the principal product which was acetylated to the diacetate *III*. However, on acetylation of the mother liquors after crystallization of the diol *II* we obtained a mixture of the 2α,3α-diacetate *III* and the 2β,3β-diacetate *VIII*. Already in our previous paper[1] we had pointed out that, contrary to the literature data[3], the hydroxylation of the 2(3)-double bond is not invariably stereospecific. We subjected the 2α,3α-diacetate *III* to the Baeyer–Villiger oxidation. Under the conditions used, we obtained, in addition to the known[2] fully acetylated brassinolide analogue *VI*, the triacetate *IV* as product of exclusive side-chain oxidation, which on alkaline hydrolysis afforded the triol *V*.

The structure of the above-mentioned 2β,3β-diacetate *VIII* was confirmed by its alkaline hydrolysis to the known[4] diol *IX*. The Baeyer–Villiger oxidation of the

* Part CCCXXVIII in the series On Steroids; Part CCCXXVII: This Journal *52*, 215 (1987).



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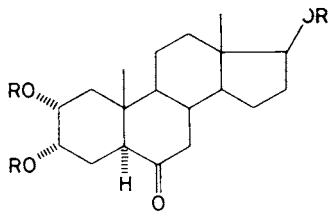

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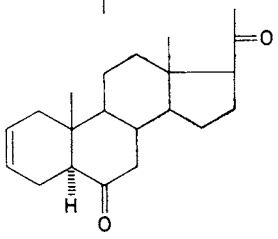

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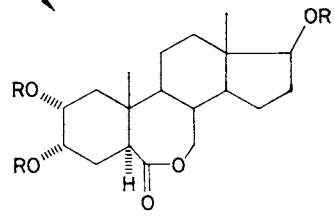

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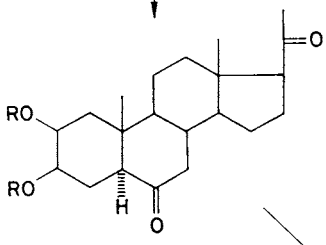

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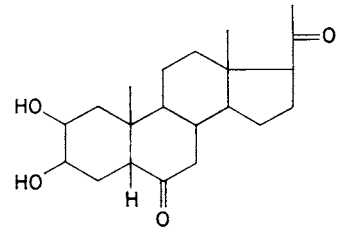
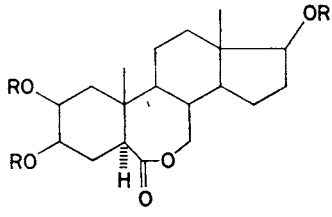
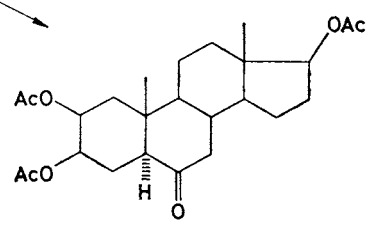


X

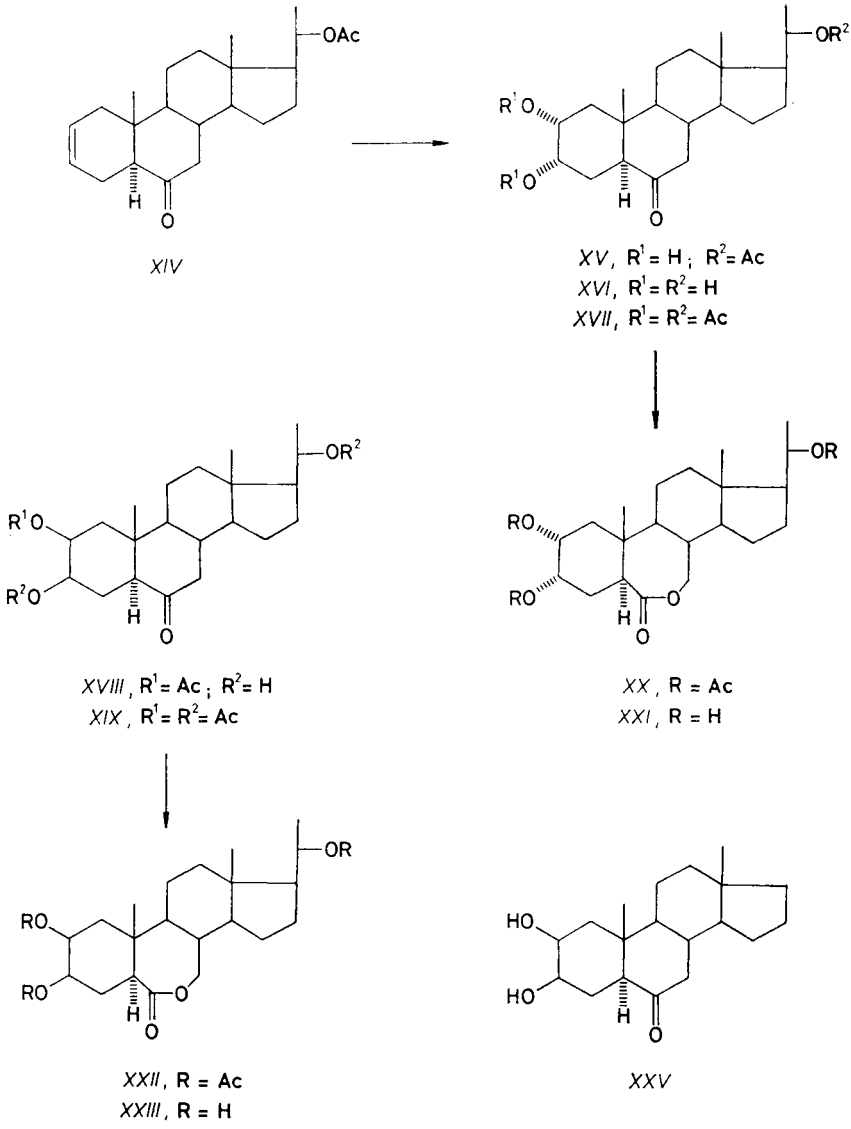


XI, R = Ac
XII, R = H



XIII

2β,3β-diacetate *VIII* proceeds analogously to that of the 2α,3α-diacetate *III* under formation of the partially oxidized product *XIII* and the fully oxidized compound *XI*. Compound *XIII* can be further oxidized to the derivative *XI*. Alkaline saponification and subsequent acidification afforded the new brassinolide analogue 2β,3β,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one (*XII*).



In the preparation of pregnane derivatives we started from the known[5] 20β--acetoxy-5α-pregn-2-en-6-one (*XIV*) which on hydroxylation with osmium tetroxide in the presence of N-methylmorpholine N-oxide furnished the diol *XV*. This, after acetylation to the triacetate *XVII*, was converted by Baeyer–Villiger oxidation to the lactone triacetate *XX*. Saponification and acidification gave the pregnane brassinolide analogue 2α,3α,20β-trihydroxy-7-oxa-B-homo-5α-pregnan-6-one (*XXI*).

Our second pregnane brassinolide analogue, 2β,3β,20β-trihydroxy-7-oxa-B-homo--5α-pregnan-6-one (*XXIII*) was obtained from the known[5] triol diacetate *XVIII via* the triacetate *XIX* which was subjected to the Baeyer–Villiger oxidation. The obtained lactone triacetate *XXII* was saponified and acidified to give the analogue *XXIII*.

The growth promoting activity of the synthetic brassino steroids and some intermediates of their synthesis was estimated by a modified bean second internode bioassay[6] (Table I). Fig. 1 shows the activity of the most potent compounds compared with that of 24-epibrassinolide *XXIV* ((22*R*, 23*R*, 24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-B-homo-5α-cholestan-6-one) as a function of the amount applied.

As follows from Table I, all the prepared brassino steroids are less active than 24-epibrassinolide *XXIV*, a very potent[7] analogue which is almost as active as natural brassinolide. Surprisingly, the most active compound in our series is 2α,3α,17β--trihydroxy-5α-androstan-6-one (*V*) whose activity nears that of epibrassinolide (Fig. 1). The already described[4] 2β,3β-dihydroxy-5α-androstan-6-one (*XXV*) showed

TABLE I

Biological activities of brassino steroids in the bean second internode bioassay

Compound	Elongation (mm) at dose (mol)							Maximal elongation at the dose, mol	24-Epibrassinolide equivalent[a] mol
	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}		
V	8·4	15·5	19·6	26·3	12·8	7·4	—	10^{-10}	$6 . 10^{-11}$
IX	9·9	6·5	5·9	8·2	5·8	0·5	—	10^{-7}	$5 . 10^{-13}$
X	9·8	7·7	9·7	4·8	12·0	10·1	—	10^{-11}	$8 . 10^{-13}$
XII	—	8·2	4·6	8·3	5·6	2·0	1·7	10^{-10}	$2 . 10^{-13}$
XXI	—	1·3	2·6	4·1	0·6	2·9	5·4	10^{-10}	$2 . 10^{-14}$
XXIII	—	7·4	3·6	5·0	3·2	1·6	0·4	10^{-8}	$1 . 10^{-14}$
XXIV	—	20·4	30·8	32·3	18·6	12·9	7·2	10^{-10}	(10^{-10})
XXV	9·5	17·5	7·3	5·3	17·4	9·9	—	10^{-11}	$8 . 10^{-12}$

[a] The amount of epibrassinolide, inducing the same elongation of the second internode as the maximal elongation induced by the tested compound.

a very interesting behaviour. This derivative, unsubstituted in the position 17, exhibits two activity maxima, the minimum between them being in the region of concentrations at which epibrassinolide shows the highest effect in the bean second internode bioassay. Of the other compounds tested, only 2β,3β-dihydroxy-5β--pregnane-6,20-dione (*X*) induces an elongation greater than 10 mm of the bean second internode; the remaining compounds are less active (Table I).

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform, error ±3°. Infrared spectra were recorded on a Zeiss UR-20 spectrometer in tetrachloromethane, unless stated otherwise. ^{1}H NMR spectra were obtained with a Tesla B 476 (60 MHz) or a Varian HA 100 (100 MHz) instrument in deuteriochloroform with tetramethylsilane as internal standard (unless stated otherwise). Chemical shifts are given in the δ-scale; $W_{1/2}$ denotes signal half-width. The mass spectra were measured on an AEI MS 902 spectrometer. The identity of the prepared samples was checked by mixture melting point determinations, thin-layer chromatography (TLC), and IR and ^{1}H NMR spectra. Preparative TLC was carried out on 200 × 200 mm plates with 0·7 mm thick layer of silica gel. "The usual work-up of the solution" means successive washing with 5% hydrochloric acid, water, 5% aqueous potassium hydrogen carbonate and water, drying over sodium sulfate, filtration and evaporation of the solvent *in vacuo*. Light petroleum was a fraction of b.p. 40–62°C.

Bean Second Internode Bioassay

Seeds of bean (*Phaseolus vulgaris*, var. PINTO) were planted in 7·0 cm clay pots containing a vermiculite with Hoagland's solution (half concentration, pH 5·7). Plants were grown in a growth room (temperature: 23 to 27°C, light: 6 000 lux for 16 h) and groups of eight 7-day-old bean seedlings with second internodes 2 mm long were treated with different concentrations (mol) of the tested compounds in 2 μl lanolin. The control plants were treated with lanolin alone. The

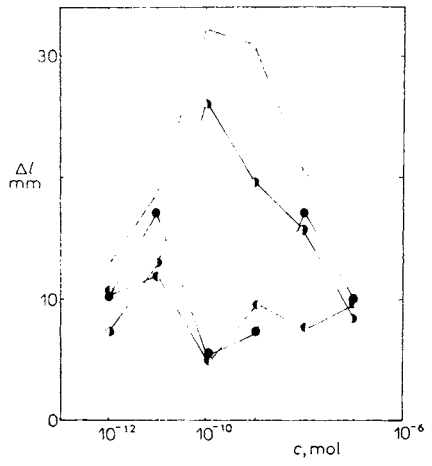


FIG. 1

Activity of brassino steroids *V* (◑), *X* (◐), *XXIV* (○), and *XXV* (●) in the bean second internode bioassay. (Δ*l* — difference, in mm, between the length of the second internode of the treated and the control plants; *c* — the amount of compound applied to one plant (in moles)

measurements were taken after 5 days. Differences in the length of the second internode of the treated and control plants (Δl in mm) were used as a measure of the activity.

2α,3α-Dihydroxy-5α-pregnane-6,20-dione (*II*)

A solution of osmium tetroxide (130 mg) in tert-butyl alcohol (1·3 ml) and a solution of N-methylmorpholine N-oxide (2·6 g) in water (4·3 ml) were added to 5α-pregn-2-ene-6,20-dione[2,8] (2·6 g) in acetone (130 ml). After stirring for 2·5 h under nitrogen, 10% sodium sulfite solution (1·5 ml) was added and the stirring was continued for 30 min. The mixture was worked up in the usual manner by extraction with chloroform. After drying and filtration through a column of charcoal and silica gel, the solvent was evaporated under diminished pressure and the residue was chromatographed on silica gel (80 g) in ether–chloroform (1 : 1). Two crystallizations from acetone–ether (1 : 10) gave 650 mg of analytically pure diol *II*, m.p. 206–211°C, $[\alpha]_D^{20}$ +62° (reported[2] m.p. 176–177°C and $[\alpha]_D^{20}$ +41·6°). IR spectrum: 3 615, 3 570 (hydroxyls), 1 709, 1 693 (C=O), 1 359 (CH_3—C=O) cm^{-1}. ^1H NMR spectrum: 0·61 and 0·74 (two s, 18-H and 19-H), 2·10 (s, 21-H), 3·68 (mt, $W_{1/2}$ = 18 Hz, 2β-H), 3·98 (mt, $W_{1/2}$ = 8·5 Hz, 3β-H).

2α,3α-Dihydroxy-5α-pregnane-6,20-dione Diacetate (*III*)

A mixture of diol *II* (3·2 g), pyridine (10 ml) and acetic anhydride (6 ml) was set aside at room temperature overnight. The mixture was worked up in the usual manner (extraction with chloroform) and the crude product was crystallized from chloroform–ether (1 : 10) to give 2·41 g of diacetate *III*, m.p. 147–153°C, $[\alpha]_D^{20}$ +47° (*c* 1·3). IR spectrum: 1 747, 1 247 (acetates), 1 712 (C=O) cm^{-1}. Mass spectrum, *m/z*: 432 (M^+). ^1H NMR spectrum (100 MHz instrument): 0·66 (s, 18-H), 0·86 (s, 19-H), 2·13 (s, 21-H), 1·99 and 2·08 (two s, 2-acetate and 3-acetate), 4·99 (mt, $W_{1/2}$ = 21 Hz, 2β-H), 5·43 (unresolved dd, J = 3 Hz, J' = 6·5 Hz, 3β-H). For $C_{25}H_{36}O_6$ (432·5) calculated: 69·42% C, 8·39% H; found: 69·70% C, 8·45% H.

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

Hydrogen peroxide (50%; 1·07 ml) was added with caution to a solution of trifluoroacetic anhydride (5·7 ml) in dichloromethane (32 ml), cooled to about 5°C. After approximately 2 min, ketone *III* (3·21 g) in dichloromethane (32 ml) was added. The mixture was allowed to stand at room temperature for 2 h and then the same solutions and amounts of trifluoroacetic anhydride and hydrogen peroxide as above were added. After standing for 4 h at room temperature, the mixture was poured into water and the product was taken up in dichloromethane. The organic phase was washed with water, potassium hydrogen carbonate solution, again with water, and dried. Evaporation of the solvent afforded a mixture containing starting ketone *III* and two compounds: one more lipophilic and the other more polar. The mixture was chromatographed on a column of silica gel (300 g) in benzene–ether (7 : 3). The lipophilic product-containing fractions gave 1·0 g of triacetate *IV*; m.p. 203–205°C, $[\alpha]_D^{20}$ −23° (*c* 1·2). IR spectrum (chloroform): 1 737, 1 259 (acetate, ketone) cm^{-1}. Mass spectrum, *m/z*: 448 (M^+). ^1H NMR spectrum: 0·79 (s, 19-H), 0·83 (s, 18-H), 1·96, 2·02 and 2·06 (three singlets, three acetates), 4·42–5·02 (mt, 2β-H and 17-H), 5·37 (dd, J = 3 Hz, J' = 6 Hz, 3β-H). For $C_{25}H_{36}O_7$ (448·5) calculated: 66·94% C, 8·09% H; found: 64·95% C, 8·08% H.

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

Potassium hydroxide (1 g) was added to a solution of triacetate *IV* (345 mg) in methanol (17 ml). After refluxing for 30 min, the mixture was concentrated under diminished pressure to about

1/4 of its original volume, poured into water and worked up in the usual manner (extraction with chloroform). Crystallization from ethanol afforded 38 mg of needles melting at 226–228°C. Evaporation of the mother liquors and crystallization of the residue from chloroform yielded further 86 mg of triol *V*, m.p. 220–225°C, $[\alpha]_D^{20}$ 0° (*c* 0·8). IR spectrum (KBr): 3 640, 1 078, 1 049, 1 021 (hydroxyls), 1 710 (C=O) cm^{-1}. ^1H NMR spectrum (100 MHz instrument, $C^2HCl_3 + C^2H_3O^2H$): 0·74 and 0·77 (two singlets, 18-H and 19-H), 3·35–3·80 (mt, 2β-H, 3β-H and 17α-H). For $C_{19}H_{30}O_4$ (322·4) calculated: 70·77% C, 9·38% H; found: 70·82% C, 9·21% H.

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

The polar product-containing fractions from the chromatography in the preparation of *IV* afforded 1·2 g of lactone *VI*, m.p. 135–138°C (ethanol), $[\alpha]_D^{20}$ +27° (*c* 2·6). IR spectrum: 1 746, 1 244 (acetates), 1 747 and 1 070 (lactone) cm^{-1}. Mass spectrum, *m/z*: 464 (M^+), 404 ($M - CH_3COOH$), 344 ($M - 2 \times CH_3COOH$). ^1H NMR spectrum: 0·83 (s, 18-H), 1·00 (s, 19-H), 1·98, 2·04 and 2·10 (three singlets, three acetates), 2·80–3·24 (mt, 5α-H), 4·07 (dm, J = 5 Hz, 7a-H), 4·45–5·06 (mt, 2β-H and 17α-H), 5·35 (br s, $W_{1/2}$ = 8 Hz, 3β-H). For $C_{25}H_{36}O_8$ (464·5) calculated: 64·63% C, 7·81% H; found: 64·51% C, 7·19% H.

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

A solution of potassium hydroxide (680 mg) in methanol (5 ml) was added to a solution of triacetate *VI* (340 mg) in methanol (10 ml). The mixture was refluxed for 60 min, cooled, acidified with hydrochloric acid and refluxed for 30 min. The solution was concentrated to about 1/3 of the original volume, poured into water and the aqueous solution was extracted with chloroform. The organic layer was separated, washed with water, dried over sodium sulfate and the solvent was evaporated. Crystallization from methanol–light petroleum afforded 109 mg of triol *VII*, m.p. 214–215°C, $[\alpha]_D^{20}$ +48° (*c* 1·5) (reported[2] m.p. 144–146°C and $[\alpha]_D^{20}$ +48° after crystallization from 95% ethanol). IR spectrum (chloroform): 3 610, 1 026 (hydroxyls), 1 722, 1 322, 1 074 (O—CO) cm^{-1}. ^1H NMR spectrum: 0·86 (s, 18-H), 0·76 (s, 19-H), 3·20–4·19 (overlapped multiplets of 5-H, 2β-H, 3β-H, 7aα-H, 7aβ-H and 17α-H).

2β,3β-Dihydroxy-5α-pregnane-6,20-dione Diacetate (*VIII*)

Mother liquors from crystallization of the 2α,3α-diol *II* (0·4 g) were dissolved in pyridine (2 ml) and allowed to stand with acetic anhydride (1 ml) overnight. The mixture was worked up as usual (extraction with chloroform) and the crude product was chromatographed on silica gel. Elution with benzene–acetone (9 : 1) afforded diacetate *III* (280 mg) and diacetate *VIII* (58 mg). Crystallization from ethanol gave 29 mg of *VIII*, m.p. 147–153°C, $[\alpha]_D^{20}$ +47° (*c* 1·3). IR spectrum: 1 747, 1 247 (acetates), 1 712 (C=O) cm^{-1}. Mass spectrum, *m/z*: 432 (M^+). ^1H NMR spectrum: 0·66 (s, 18-H), 0·86 (s, 19-H), 2·13 (s, 21-H), 1·99 and 2·08 (two singlets, 2-acetate and 3-acetate), 4·99 (mt, $W_{1/2}$ = 21 Hz, 2β-H), 5·43 (unresolved dd, J = 3 Hz, J' = 6·5 Hz, 3β-H). For $C_{25}H_{36}O_6$ (432·5) calculated: 69·42% C, 8·39% H; found: 69·70% C, 8·45% H.

2β,3β-Dihydroxy-5α-pregnane-6,20-dione (*IX*)

Potassium hydroxide (200 mg) was added to a solution of diacetate *VIII* (62 mg) in methanol (10 ml). After standing at room temperature overnight, the solution was evaporated and the product was extracted with chloroform and processed in the usual manner. The evaporation residue (42 mg) was separated by preparative chromatography on two plates of silica gel (elution

with chloroform–ether 1 : 1). Work-up of zones containing the lipophilic product furnished 10 mg of diol *IX*, m.p. 192–194°C, $[\alpha]_D^{20}$ +48° (*c* 1·1), in accord with the literature[4] (m.p. 193–194°C, $[\alpha]_D^{20}$ +49·5°).

2β,3β-Dihydroxy-5β-pregnane-6,20-dione (*X*)

Polar product-containing zones from the chromatography in the preceding experiment afforded 28 mg of diol *X*, m.p. 154–155°C, $[\alpha]_D^{20}$ −19° (*c* 1·1) (reported[4] m.p. 154–155°C, $[\alpha]_D^{20}$ −18°). ^{1}H NMR spectrum: 0·62 (s, 18-H), 0·90 (s, 19-H), 2·11 (s, 21-H), 2·24 (br s, J = 7 Hz, 7-H), 3·20–4·27 (overlapped broad and narrow multiplets, 3α-H and 2α-H).

2β,3β,18β-Trihydroxy-7-oxa-B-homo-5α-androstan-6-one Triacetate (*XI*)

A) Chromatographic fractions in the preparation of *XIII* afforded 43 mg of lactone *XI*, m.p. 248–250°C (methanol), $[\alpha]_D^{20}$ +54° (*c* 0·5). IR spectrum (chloroform): 1 735, 1 225, 1 022 (acetates), 1 728, 1 053 (lactone) cm^{-1}. Mass spectrum, *m/z*: 464 (M^+). CD spectrum (dioxane): $\Delta\varepsilon_{224}$ = +1·6. ^{1}H NMR spectrum (200 MHz instrument): 0·82 (s, 18-H), 1·05 (s, 19-H), 2·02, 2·04 and 2·09 (three singlets, three acetates), 2·31 (dd, J_{gem} = 15·6 Hz, $J_{4\alpha,5\alpha}$ = 4 Hz, 4α-H), 2·53 (d, J_{gem} = 15·6 Hz, $J_{4\beta,5\alpha}$ = 12·3 Hz, 4β-H), 4·02–4·07 (mt, 7a-H), (4·60 dd, $J_{17\alpha,16\alpha}$ + $J_{17\alpha,16\beta}$ = 7·3 Hz and 9·3 Hz, 17α-H), 4·80 (ddd, 3α-H, $J_{3\alpha,4\beta}$ = 13·1 Hz, $J_{3\alpha,4\alpha}$ = 4·0 Hz, $J_{3\alpha,2\alpha}$ = 3·0 Hz), 5·83 (mt, $\sum J$ = 10 Hz, 2α-H). For $C_{25}H_{36}O_8$ (464·54) calculated: 64·63% C, 7·81% H; found: 64·81% C, 7·92% H.

B) A solution of ketone *XIII* (300 mg) in dichloromethane (4 ml) was added at 0°C to a stirred solution of trifluoroacetic anhydride (0·8 ml) and hydrogen peroxide (50%; 0·16 ml) in dichloromethane (10 ml). After standing at room temperature for 24 h, the mixture was poured into ice-cold 5% aqueous solution of potassium hydrogen carbonate and the product was taken up in ethyl acetate. The extract was washed with water, dried over sodium sulfate and the solvent was evaporated *in vacuo*. The residue (295 mg) was preparatively chromatographed on 6 plates of silica gel in light petroleum–acetone (1 : 1), affording 235 mg of product which on crystallizafrom acetone–heptane gave 150 mg of lactone *XI*, m.p. 248–250°C, $[\alpha]_D^{20}$ +54° (*c* 0·5).

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

A mixture of triacetoxy derivative *XI* (50 mg), potassium carbonate (150 mg) and 70% methanol (7·5 ml) was refluxed for 2 h. After cooling, the mixture was diluted with tetrahydrofuran (10 ml), acidified with 6M-HCl and boiled for 30 min. The acid was neutralized with 5% aqueous potassium hydrogen carbonate and the product was extracted with ethyl acetate. The organic extract was washed with water, dried and the solvent was evaporated. The residue (40 mg) was purified by preparative chromatography on a plate of silica gel in chloroform–ether–methanol (7 : 2 : 1). The obtained product (30 mg) was crystallized from methanol–ethyl acetate to afford 17 mg of triol *XII*, m.p. 292–294°C. IR spectrum (KBr): 3 450 (hydroxyls), 1 723, 1 705, 1 794, 1 333, 1 238 (lactone), 1 086, 1 060, 1 040, 1 016 (hydroxyls and lactone) cm^{-1}. For $C_{19}H_{30}O_5$ (338·4) calculated: 67·43% C, 8·94% H; found: 67·08% C, 8·67% H.

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

m-Chloroperoxybenzoic acid (178 mg) was added to a solution of diketone *VIII* (178 mg) in dichloromethane (3·5 ml), the mixture was set aside at room temperature for 7 days and then poured into water. The product was extracted with dichloromethane, washed with 5% aqueous potassium hydrogen carbonate, water, and dried. The solvent was evaporated, leaving 153 mg

of material which was chromatographed on 4 plates of silica gel in light petroleum–acetone (3 : 2). Work-up of zones, containing the lipophilic product, yielded 69 mg of triacetate *XIII*, m.p. 215–217°C (methanol), $[\alpha]_D^{20}$ −10° (*c* 0·5). IR spectrum: 1 743, 1 247 (acetates and ketone) cm^{-1}. Mass spectrum, *m/z*: 448 (M^+). ^1H NMR spectrum: 0·78 (s, 18-H), 0·93 (s, 19-H), 1·98, 2·03 and 2·05 (three singlets, three acetates), 4·39–5·00 (2 mt, 3-H and 17-H), 5·27 (mt, $W_{1/2}$ = = 5 Hz, 2α-H). For $C_{25}H_{36}O_7$ (448·54) calculated: 66·94% C, 8·09% H; found: 67·01% C, 8·08% H.

2α,3α,20β-Trihydroxy-5α-pregnan-6-one 20-Acetate (*XV*)

Osmium tetroxide in tert-butyl alcohol (10% solution; 0·15 ml), tert-butyl alcohol (0·35 ml), water (0·5 ml) and N-methylmorpholine N-oxide (300 mg) were successively added to a solution of 20β-acetoxy-5α-pregn-2-en-6-one[5] (*XIV*; 300 mg) in acetone (15 ml). After stirring in an atmosphere of nitrogen at room temperature for 24 h, the excess osmium tetroxide was decomposed with 3% sodium thiosulfate and the product was taken up in chloroform. The extract was washed with 5% hydrochloric acid, saturated sodium chloride solution, dried and the solvent was evaporated *in vacuo*. The residue (300 mg) was mixed with ether to give 255 mg of diol *XV* which was crystallized from acetone–heptane, m.p. 216–218°C; yield 200 mg. IR spectrum (chloroform): 3 620 (hydroxyls), 1 721, 1 260 (acetates), 1 713 (ketone), 1 076, 1 052, 1 043 (hydroxyls and acetate) cm^{-1}. For $C_{23}H_{36}O_5$ (392·5) calculated: 70·37% C, 9·25% H; found: 70·01% C, 9·04% H.

2α,3α,20β-Trihydroxy-5α-pregnan-6-one (*XVI*)

A mixture of acetate *XV* (100 mg), potassium carbonate (200 mg) and 70% methanol (15 ml) was refluxed for 5 h and then poured into water. The product was extracted with chloroform, the extract was washed with water, dried over sodium sulfate and taken down. The residue (95 mg) was chromatographed on 2 plates of silica gel in chloroform–ether (7 : 3), affording 50 mg of triol *XVI*, m.p. 224–226°C (acetone–heptane). IR spectrum (KBr, micropellet): 1 708 (ketone), 3 410 (hydroxyls) cm^{-1}. CD spectrum (dioxane): λ_{max} 301 nm, $\Delta\varepsilon$ −1·4. For $C_{21}H_{34}O_4$ (350·5) calculated:71·96% C, 9·78% H; found:71·68% C, 9·57% H.

2α,3α,20β-Trihydroxy-5α-pregnan-6-on Triacetate (*XVII*)

A mixture of diol *XV* (200 mg), pyridine (6 ml) and acetic anhydride (4 ml) was set aside for 2 days. The usual work-up procedure afforded 200 mg of crude product which on crystallization from acetone–heptane afforded 157 mg of the triacetate *XVII*, m.p. 210–211°C. IR spectrum (chloroform): 1 737, 1 725, 1 259 (acetate),1 711 (ketone) cm^{-1}. ^1H NMR spectrum: 0·61 (s, 18-H), 0·80 (s, 19-H), 1·15 (d, J = 6 Hz, 21-H), 1·95, 1·98 and 2·05 (three singlets, three acetates), 4·88 (mt, 2β-H and 3β-H), 5·35 (mt, 20α-H). For $C_{27}H_{40}O_7$ (476·6) calculated: 68·04% C, 8·46% H; found: 67·89% C, 8·27% H.

2β,3β,20β-Trihydroxy-5α-pregnan-6-one Triacetate (*XIX*)

A mixture of 2β,3β,20β-trihydroxy-5α-pregnan-6-one 2-acetate[5] (*XVIII*; 300 mg), acetic anhydride (4 ml) and pyridine (8 ml) was allowed to stand overnight. The usual work-up procedure afforded 300 mg of crude product which was crystallized from methanol to give the title triacetate *XIX* (235 mg), m.p. 244–246°C. IR spectrum (chloroform): 1 735, 1 726, 1 260 (acetates), 1 711 (ketone) cm^{-1}. For $C_{27}H_{40}O_7$ (476·6) calculated: 68·04% C, 8·46% H; found: 67·99% C, 8·35% H.

2α,3α,20β-Trihydroxy-7-oxa-B-homo-5α-pregnan-6-one Triacetate (*XX*)

A solution of ketone *XVII* (80 mg) in dichloromethane (1 ml) was oxidized with trifluoroperoxyacetic acid (prepared from 0·14 ml of trifluoroacetic anhydride and 0·02 ml of 50% hydrogen peroxide). After standing for 4 h at room temperature, the mixture was worked up as described in the preparation of *XI*, procedure *B*), affording 60 mg of lactone *XX*, m.p. 254–256°C (acetone–heptane). IR spectrum (chloroform): 1 730, 1 256, 1 048, 1 027 (acetates, lactone) cm^{-1}. ^1H NMR spectrum (200 MHz instrument): 0·68 (s, 18-H), 0·98 (s, 19-H), 1·16 (s, $J = 6·2$ Hz, 21-H), 1·99, 2·01 and 2·12 (three singlets, three acetates), 2·30 (ddd, $J_{gem} = 15·8$ Hz, $J_{4\beta,5\alpha} = 12·4$ Hz, $J_{4\beta,3\beta} = 2·5$ Hz, 4β-H), 3·00 (dd, $J_{5\alpha,4\alpha} = 4·6$ Hz, $J_{5\alpha,4\beta} = 12·4$ Hz, 5α-H), 4·81–4·92 (mt, 2β-H and 20α-H), 5·37 (mt, $\sum J = 10$ Hz, 3β-H), 4·02–4·12 (mt, 7a-H). CD spectrum (dioxane): λ_{max} 220 nm, $\Delta\varepsilon$ +3·1. For $C_{27}H_{40}O_8$ (492·6) calculated: 65·83% C, 8·19% H; found: 65·66% C, 8·01% H.

2α,3α,20β-Trihydroxy-7-oxa-B-homo-5α-pregnan-6-one (*XXI*)

Triacetate *XX* (50 mg) was hydrolyzed with potassium carbonate (160 mg) in 70% methanol (8 ml) in the same manner as described in the preparation of *XII*. The crude product (45 mg) was chromatographed on one plate of silica gel in a chloroform–light petroleum–methanol (8 : 1 : 1) mixture. Work-up of the corresponding zone afforded product (30 mg) which was purified by crystallization from acetone–heptane to give 20 mg of triol *XXI*, m.p. 236–239°C. IR spectrum (chloroform): 3 615 (hydroxyls), 1 724, 1 251 (lactone), 1 069, 1 046, 1 028 (lactone and hydroxyls) cm^{-1}. For $C_{21}H_{34}O_5$ (366·5) calculated: 68·82% C, 9·35% H; found: 68·56% C, 9·17% H.

2β,3β,20β-Trihydroxy-7-oxa-B-homo-5α-pregnan-6-one Triacetate (*XXII*)

A solution of ketone *XIX* (150 mg) in dichloromethane (2 ml) was added at 0°C to a stirred solution of trifluoroacetic anhydride (0·4 ml) and 50% hydrogen peroxide (0·08 ml) in dichloromethane (5 ml). After standing for 5 h at room temperature, the mixture was worked up as described for the preparation of lactone *XI* according procedure *B*). The crude product (150 mg) was purified by preparative chromatography on 3 plates of silica gel in light petroleum–ethyl acetate (6 : 4; threefold developing). The corresponding zones were combined, the product was washed out with ether and the solvent was evaporated *in vacuo*. Crystallization of the residue (135 mg) from acetone–heptane afforded 100 mg of lactone *XXII*, m.p. 281–284°C. IR spectrum (chloroform): 1 729, 1 259 (acetates), 1 729, 1 070 (lactone) cm^{-1}. ^1H NMR spectrum (200 MHz): 0·66 (s, 18-H), 1·04 (s, 19-H), 1·15 (d, $J = 6·2$ Hz, 21-H), 2·01, 2·02 and 2·09 (three singlets, three acetates), 2·30 (dd, $J_{gem} = 15·1$ Hz, $J_{4\alpha,5\alpha} = 3·9$ Hz, 4α-H), 2·53 (ddd, $J_{gem} = 15·1$ Hz, $J_{4\beta,5\alpha} = 12·3$ Hz, 4β-H), 4·75–4·88 (mt, 5α-H and 20α-H), 5·23 (mt, 2α-H). CD spectrum (dioxane): λ_{max} 220 nm, $\Delta\varepsilon$ +2·7. For $C_{27}H_{40}O_8$ (492·6) calculated: 65·83% C, 8·19% H; found: 65·71% C, 8·09% H.

2β,3β,20β-Trihydroxy-7-oxa-B-homo-5α-pregnan-6-one (*XXIII*)

Triacetate *XXII* (60 mg) was hydrolyzed with potassium carbonate (170 mg) in 70% methanol (10 ml) as described for the preparation of compound *XII*. Crystallization from acetone–heptane–methanol gave 23 mg of triol *XXIII*, m.p. 300–303°C. IR spectrum (KBr): 3 530, 3 310, 1080 (hydroxyls), 1 710, 1 328, 1 196 (lactone) cm^{-1}. ^1H NMR spectrum (200 MHz, $C^2H_3O^2H$): 0·79 (s, 18-H), 1·03 (s, 19-H), 1·10 (d, $J = 6·2$ Hz, 21-H), 2·21 (dd, $J_{gem} = 15·0$ Hz, $J_{4\alpha,5\alpha} = 3·9$ Hz, 4α-H), 2·38 (dd, $J_{gem} = 15$ Hz, $J_{4\beta,5\alpha} = 12·4$ Hz, 4β-H), 3·53–3·68 (mt, 3α-H and

20α-H), 4·03 (dd, J_{gem} = 12·7 Hz, $J_{7\alpha,8\alpha}$ = 2·0 Hz, 7α-H), 4·19 (dd, J_{gem} = 12·7 Hz, $J_{7\beta,8\alpha}$ = = 9·1 Hz, 7β-H), CD spectrum (dioxane): λ_{max} 223 nm, $\Delta\varepsilon$ +1·9. For $C_{21}H_{34}O_5$ (366·5) calculated: 68·82% C, 9·35% H; found: 68·54% C, 9·13% H.

The analyses were performed in the Analytical Laboratory of this Institute by Mrs E. Sýkorová, V. Rusová and E. Šípová under supervision of Dr J. Horáček. IR spectra were recorded by Mrs K. Matoušková and Miss H. Kapičková and interpreted by Dr J. Smolíková. CD spectra were measured by Dr S. Vašíčková, mass spectra by Mrs P. Loudová. Measurements of ^{1}H NMR *spectra were carried out by Mrs J. Jelínková and Mrs M. Snopková, spectra on the 200 MHz instrument were taken by Dr J. Zajíček. The technical assistance of Mrs J. Mašková is gratefully acknowledged.*

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Translated by M. Tichý.