BRASSINOSTEROIDS WITH ESTER FUNCTION WITH FIVE CARBON ATOMS AT THE 20 POSITION*

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Pregnane analogues of brassinolide and castasterone have been prepared which have an ester function with five carbon atoms at the 20 position. When tested by the bean second internode bioassay, the most efficient substance is *VIII*.

In one of our previous papers dealing with the structure–activity relationships of brassinosteroids[1] we prepared androstane analogues of brassinolide with an ester function with five carbon atoms at the 17 position. These compounds exhibited a high brassinolide activity. Therefore we decided to prepare similar esters of pregnane derivatives which are structurally closer to brassinolide.

When preparing brassinosteroids with an ester function one can adopt the following procedure: the preparation of the steroid part with brassinolide structure, i.e. 2α,3α-dihydroxy-6-keto-7-oxa-, or with castosterone structure, i.e. 2α,3α-dihydroxy-6-keto-, followed by introduction of the ester function into the molecule. Or, alternatively, the ester function is introduced first, and then the brassinolide structure is formed in the rings A and B.

In the first case we started from the known[2] 2α,3α-dihydroxy-5α-pregnane-6,20-dione (*I*). Its reduction gave the (20*R*)-hydroxy derivative *II*. The 2α,3α-diol grouping was protected as the acetonide *V*, whereafter the respective ester (3-methylbutyrate *VI* or 2-methylbutyrate *VII*) was prepared by reaction with chloride of 3-methylbutyric or 2-methylbutyric acid in pyridine. The protecting group on the ring A was removed to give the castosterone analogues (20*R*)-2α,3α,20-trihydroxy-5α-pregnan-6-one 20-(3-methylbutyrate) (*III*) and (20*R*)-2α,3α,20-trihydroxy-5α-pregnan-6-one 20-(2-methylbutyrate) (*IV*). The Baeyer–Villiger oxidation with trifluoroperacetic acid

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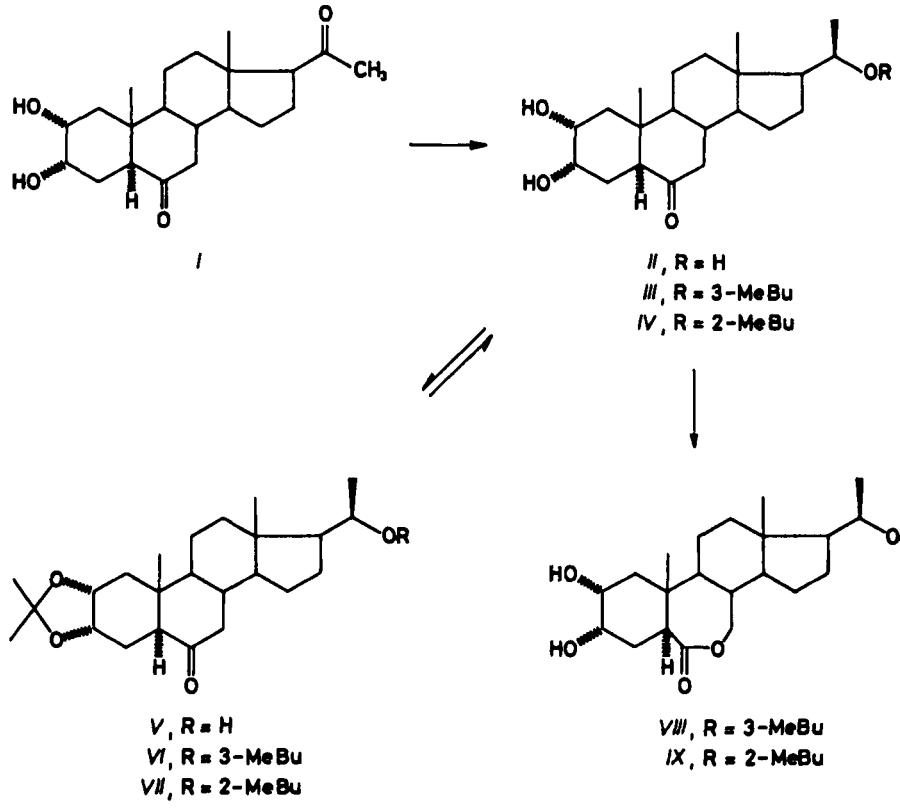
in dichloromethane converted the two ketones (*III* and *IV*) into the corresponding brassinolide analogues: the lactones *VIII* and *IX*.



I

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

V, R = H
VI, R = 3-MeBu
VII, R = 2-MeBu

VIII, R = 3-MeBu
IX, R = 2-MeBu

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

In the second case we started from (20*R*)-pregn-5-ene-3α,20-diol 3-(4-methylbenzenesulfonate)[3] (*X*), which reacted with 2-methylbutanoyl chloride to give the 2-methylbutyrate *XI*. Its rearrangement induced by potassium acetate gave the 6-hydroxy derivative *XII* which was oxidized to the 6-ketone *XIII* which gave olefin *XIV* by treatment with lithium bromide in N,N-dimethylacetamide in the presence of pyridinium 4-methylbenzenesulfonate. The hydroxylation of double bond in the olefin *XIV* gave the above-mentioned required diol *IV* – an analogue of castasterone.

Comparison of the two preparative pathways starting from pregnenolone acetate shows that the second one is more advantageous being shorter by three steps.

The biological activity of the compounds prepared (viz. *III* – *X* and *XIV*) was tested by the bean second internode bioassay[4]. From among all the substances tested the

highest activity was exhibited by the compound *VIII*: (20*R*)-2α,3α,20-trihydroxy-7-oxa-B-homo-5α-pregnan-6-one 20-(3-methylbutyrate). The other brassinolide analogue (*IX*) and both castosterone analogues (*III* and *IV*) were substantially less active (Table I).

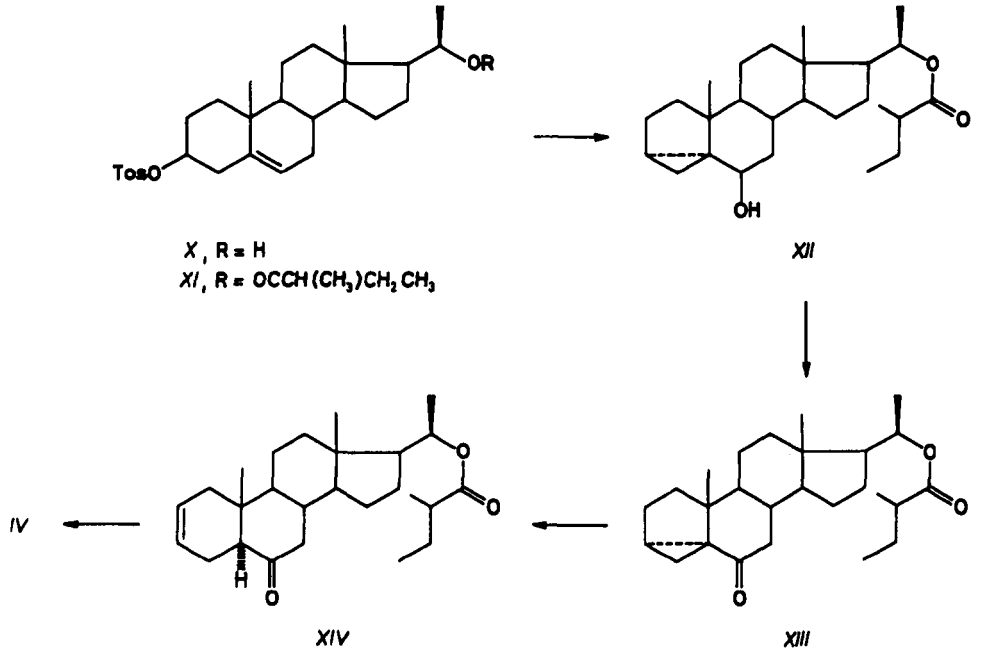


TABLE I
Results of testing of compounds *III* – *V* and *VII* – *IX* by the bean second internode bioassay[4]

Compound	Lengthening of the second internode [a], mm	The amount applied [b], mol
III	3.2	$1 . 10^{-9}$
IV	9.1	$1 . 10^{-9}$
V	13.4	$1 . 10^{-8}$
VII	11.3	$1 . 10^{-8}$
VIII	16.2	$1 . 10^{-8}$
IX	4.9	$1 . 10^{-8}$

[a] The lengthening of the second internode means its lengthening as compared with that of the reference plant for the applied amount given. [b] The applied amount means the amount causing the maximum lengthening of the second internode (all the substances tested were applied in the amounts from $1 . 10^{-12}$ to $1 . 10^{-7}$ mol per one plant).

Interestingly, the acetonides *V* and *VII* exhibit a certain activity. The other substances tested (*VI*, *X*, and *XIV*) were practically inactive in this test, at some concentrations they even retarded the growth of plants as compared with the reference plants.

EXPERIMENTAL

The melting points were estimated with a Kofler apparatus and are not corrected. If not otherwise stated, the IR spectra were measured with a Zeiss UR 20 apparatus in tetrachloromethane. The wavenumbers are given in cm^{-1}. The ^1H NMR spectra were measured with a Tesla BS 497 spectrometer (100 MHz) in deuteriochloroform with tetramethylsilane as the internal standard. The chemical shifts are given in ppm (δ scale), the coupling constants (J) and half-widths of multiplets ($W_{1/2}$) are given in Hz. The symbol $W_{1/2}$ means the width of signal at its half height. The spectra were interpreted as the first-order spectra. The mass spectra were measured with a ZAB-EG spectrometer at 70 eV.

The identity of the samples prepared was checked by mixed melting points, TLC, IR spectra, and ^1H NMR spectra. For preparative TLC we adopted 200 × 200 mm plates with silica gel layer (Woelm DC) of 0.7 mm thickness.

The term "usual working-up of solution" means washing of the solution with 5% hydrochloric acid, water, 5% aqueous solution of potassium hydrogen carbonate, water, drying over sodium sulfate, removal of sodium sulfate by filtration, and removal of solvent by distillation until dry in vacuum. If petroleum ether is mentioned, then it means its fraction boiling from 40 to 62 °C.

(20*R*)-2α,3α,20-Trihydroxy-5α-pregnan-6-one (*II*)

A solution of 100 mg (0.29 mmol) of 2α,3α-dihydroxy-5α-pregnan-6,20-dione (*I*), m.p. 207 – 210 °C, in 10 ml tetrahydrofuran was treated with 200 mg (0.79 mmol) of lithium tri-tert-butoxyaluminohydride. The reaction mixture was left to stand at room temperature 1 h, whereafter it was carefully poured into water and the product was processed by ether extraction as usual. The evaporation residue contained mainly a single product and was submitted to preparative TLC using four silica gel plates and chloroform–ether–methanol 4 : 4 : 1 as the eluent. The processing of the corresponding zones gave 81 mg of product which was recrystallized from a mixture of acetone and heptane to give 62 mg (61%) of triol *II*, m.p. 215 – 218 °C (in accordance with ref.[2]). IR spectrum (chloroform): 3 620 (O–H); 1 711 (C=O); 1 107, 1 056, 1 047 (O–H). ^1H NMR spectrum: 0.75 s, 6 H (3 × H-18 and 3 × H-19); 1.16 d, 3 H (3 × H-21, J = 6); 3.74 m, 2 H (H-2β and H-20, $W_{1/2}$ = 23); 4.00 m, 1 H (H-3β, $W_{1/2}$ = 5). For $C_{21}H_{34}O_4$ (350.5) calculated: 71.96% C, 9.78% H; found: 71.86% C, 9.80% H.

(20*R*)-2α,3α,20-Trihydroxy-5α-pregnan-6-one 20-(3-Methylbutyrate) (*III*)

A solution of 330 mg (0.70 mmol) of isopropylidenedioxy derivative *VI* in 20 ml of methanol was treated with 0.1 ml (0.36 mmol) of 37% hydrochloric acid. The mixture was shaken at room temperature 30 min, concentrated to about 1/4 volume, and poured into water. The product was extracted with chloroform, the chloroform layer was separated and washed with saturated solution of potassium hydrogen carbonate, with water, dried over anhydrous sodium sulfate, and the solvent was distilled off in vacuum. Yield 320 mg of product which was purified by column chromatography (silica gel, petroleum ether–chloroform–ether 2 : 1 : 1) to give 213 mg (70%) of 3-methylbutyrate *III*, m.p. 77 – 78 °C (ethanol–water). IR spectrum: 3 450 (O–H); 1 735, 1 198 (COOR); 1 700 sh (C=O, ketone); 1 126, 1 100, 1 080, 1 058 (C–O). ^1H NMR spectrum: 0.63 s, 3 H (3 × H-18); 0.74 s, 3 H (3 × H-19); 1.15 d, 3 H (H-21, J = 6); 0.96 d, 6 H ($(CH_3)_2CH$ of the ester group, J = 6.0); 3.74 m, 1 H (H-2β, $W_{1/2}$ = 23); 4.02 m, 1 H (H-3β, $W_{1/2}$ = 8.5);

4.84 m, 1 H (H-20, $W_{1/2}$ = 20). For $C_{26}H_{42}O_5$ (434.6) calculated: 71.85% C, 9.74% H; found: 72.05% C, 9.81% H. Mass spectrum (*m/z*): 434 (M^+).

(20*R*)-2α,3α,20-Trihydroxy-5α-pregnan-6-one 20-(2-Methylbutyrate) (*IV*)

A) A solution of 60 mg (0.13 mmol) of *VII* in 20 ml of methanol was treated with 0.5 ml (0.18 mmol) of 37% hydrochloric acid, the mixture was heated to boiling and left to stand without further heating 1 h. Then it was poured into water and processed in usual way by extraction with ether to give 55 mg of evaporation residue which was purified by TLC on three silica gel plates (chloroform–petroleum ether–ether 1 : 1 : 2). Yield 41 mg (75%) of ester *IV*. Its difficult recrystallization from aqueous ethanol gave 8 mg of product *IV*, m.p. 78 – 81 °C. IR spectrum: 3 620 (O–H); 1 728, 1 193 (COOR); 1 710 sh (C=O); 1 083, 1 000 sh (C–O). ^{1}H NMR spectrum: 0.63 s, 3 H (3 × H-18); 0.74 s, 3 H (3 × H-19); 1.14 d, 3 H (H-21, *J* = 7); 1.22 d, 3 H (CH_3CH of the ester group, *J* = 7); 0.91 t, 3 H (CH_3CH_2 of the ester group, *J* = 7); 3.70 m, 1 H (H-2β); 4.02 m, 1 H (H-3β, $W_{1/2}$ = 9); 4.81 m, 1 H (H-20, $W_{1/2}$ = 17). For $C_{26}H_{42}O_5$ (434.6) calculated: 71.85% C, 9.74% H; found: 71.45% C, 9.42% H.

B) A solution of 5.3 g (13.2 mmol) of olefin *XIV* in 265 ml of acetone was treated with a solution of 265 mg (1.04 mmol) of osmium tetroxide in 2.65 ml of tert-butyl alcohol, whereupon 6.25 ml of tert-butyl alcohol, 5.3 g (45.2 mmol) of N-methylmorpholine-N-oxide and 8.8 ml of water were added. The mixture was stirred at room temperature 4 h. Then 10 ml 10% sodium sulfite was added and the stirring at room temperature was continued for another 30 min. The reaction mixture was poured into water, the product was extracted with chloroform, the organic layer was separated, washed with water, dried with anhydrous sodium sulfate, and the solvent was distilled off. The evaporation residue was purified by column chromatography (250 g silica gel, petroleum ether–ether 1 : 1, then benzene–ether 1 : 1, and finally benzene–ether–methanol 10 : 10 : 1) to give 5.5 g (96%) of triol *IV* identical with the substance prepared by the above procedure *A*.

(20*R*)-2α,3α-Isopropylidenedioxy-20-hydroxy-5α-pregnan-6-one (*V*)

A solution of 200 mg (0.57 mmol) of triol *II* in 20 ml of acetone was treated with 20 mg (0.11 mmol) of 4-methylbenzenesulfonic acid monohydrate and 200 mg (1.3 mmol) of anhydrous copper(II) sulfate. The mixture was stirred at room temperature 72 h, whereafter it was poured into a saturated solution of potassium hydrogen carbonate, and the product was extracted with ether. The ethereal layer was washed with water, dried with anhydrous sodium sulfate, and the solvent was distilled off to give 210 mg of evaporation residue which was purified by TLC (eight silica gel plates, chloroform–ether 1 : 1). Yield 146 mg (66%) isopropylidenedioxy derivative *V*, m.p. 229 – 233 °C (subl. about 190 °C). ^{1}H NMR spectrum: 0.73 s, 3 H (3 × H-18); 0.67 s, 3 H (3 × H-19); 1.15 d, 3 H (3 × H-21, *J* = 6.5); 1.33 s, 2 × 3 H (2 × CH_3 of the acetonide); 3.73 dq, 1 H (H-20α, *J* = 6.5; *J'* = 11); 3.94 – 4.34 m, 2 H (H-2β and H-3β). For $C_{24}H_{38}O_4$ (390.5) calculated: 73.81% C, 9.81% H; found: 73.95% C, 9.79% H.

(20*R*)-2α,3α-Isopropylidenedioxy-20-hydroxy-5α-pregnan-6-one 20-(3-Methylbutyrate) (*VI*)

A solution of 2 g (5.1 mmol) of alcohol *V* in 100 ml of pyridine was treated with a fresh solution of 4 g (3.3 mmol) of 3-methylbutanoyl chloride in 40 ml of pyridine. The reaction mixture was left to stand at room temperature overnight. Then it was poured into a mixture of ice and water, and the product was processed by extraction with ether as usual. The evaporation residue (2 g) was purified by column chromatography (250 g silica gel, petroleum ether–chloroform–ether 2:1:1). Processing of the respective fractions gave 1.7 g of evaporation residue which was recrystallized from methanol to give 1.2 g (50%) of crystalline product, m.p. 189 – 191 °C. ^{1}H NMR spectrum: 0.63 s, 3 H (3 × H-18); 0.67 s, 3 H (3 × H-19); 1.16 d, 3 H (3 × H-21, *J* = 6); 0.97 d, 6 H ($(CH_3)_2CH$ of the ester group, *J* = 7); 1.35 s and

1.50 s, 2 × 3 H (2 × CH_3 of the acetonide); 4.10 m, 1 H (H-2β, $W_{1/2}$ = 26); 4.25 m, 1 H (H-3β, $W_{1/2}$ = 12); 4.84 dq, 1 H (H-20, J = 11; J' = 6). Mass spectrum (m/z): 459 (M – CH_3). For $C_{29}H_{46}O_5$ (474.7) calculated: 73.38% C, 9.77% H; found: 74.15% C, 9.87% H.

(20*R*)-2α,3α-Isopropylidenedioxy-20-hydroxy-5α-pregnan-6-one 20-(2-Methylbutyrate) (*VII*)

A solution of 110 mg (0.28 mmol) of alcohol *V* in 3 ml of pyridine was treated with a fresh solution of 0.1 ml (1.00 mmol) of 2-methylbutanoyl chloride in 0.6 ml of pyridine. The mixture was left to stand at room temperature 48 h, whereafter it was poured into a mixture of ice and water and treated in usual way by extraction with ether. Yield 120 mg of oil which was purified by column chromatography (100 g silica gel, petroleum ether–ether–chloroform 10 : 10 : 1). The work-up of corresponding fractions (evaporation of solvent and recrystallization of the evaporation residue (89 mg) from aqueous ethanol) gave 22 mg (16%) of ester *VII*, m.p. 142 – 144 °C. IR spectrum (KBr): 1 728, 1 188 (COOR); 1 700 sh (C=O ketone); 1 261 (acetonide). ^{1}H NMR spectrum: 0.62 s, 3 H (3 × H-18); 0.67 s, 3 H (3 × H-19); 0.89 t, 3 H (CH_3CH_2 of the ester group, J = 7); 1.14 d, 3 H (3 × H-21, J = 7); 1.22 d, 3 H (CH_3CH of the ester group, J = 7); 1.34 s and 1.50 s, 2 × 3 H (2 × CH_3 of the acetonide); 3.90 – 4.35 m, 2 H (H-2β and H-3β); 4.84 m, 1 H (H-20, $W_{1/2}$ = 10). Mass spectrum (m/z): 459 (M – CH_3). For $C_{29}H_{46}O_5$ (474.7) calculated: 73.38% C, 9.77% H; found: 73.48% C, 9.83% H.

(20*R*)-2α,3α,20-Trihydroxy-7-oxa-B-homo-5α-pregnan-6-one 20-(3-Methylbutyrate) (*VIII*)

A solution of 130 mg (0.3 mmol) of ketone *III* in 13 ml of dichloromethane was treated with a solution trifluoroperacetic acid prepared from 0.9 g (4.3 mmol) of trifluoroacetic acid anhydride and 0.14 g (2.1 mmol) of 50% hydrogen peroxide in 1.9 ml of dichloromethane. The mixture was left to stand at room temperature 24 h, whereafter it was poured into 10% solution of potassium hydrogen carbonate, washed with water, dried with anhydrous sodium sulfate, and the solvent was distilled off. The evaporation residue (128 mg) was purified by TLC (eight silica gel plates, chloroform–ether 1 : 1). The work-up of corresponding zones gave 46 mg (34%) of lactone *VIII*, m.p. 121 – 124 °C (aqueous ethanol). IR spectrum: 3 620 (O–H); 1 730 (C=O); 1 198, 1 090, 1 077 (C–O). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.16 d, 3 H (3 × H-21, J = 6.5); 0.96 d, 6 H ($(CH_3)_2CH$ of the ester, J = 7.5); 3.74 m, 1 H (H-2β, $W_{1/2}$ = 26); 4.05 d, 2 H (H-7a, J = 5; overlapping multiplet H-3β, 1 H); 4.85 m, 1 H (H-20, $W_{1/2}$ = 18). Mass spectrum (m/z): 432 (M – H_2O). For $C_{26}H_{42}O_6$ (450.6) calculated: 69.30% C, 9.40% H; found: 69.19% C, 9.45% H.

(20*R*)-2α,3α-Dihydroxy-7-oxa-B-homo-5α-pregnan-6-one 20-(2-Methylbutyrate) (*IX*)

A solution of 0.8 g (1.8 mmol) of 6-ketone *IV* in 25 ml of dichloromethane was treated with a fresh solution of trifluoroperacetic acid prepared from 5.496 g (26 mmol) of trifluoroacetic acid anhydride, 0.86 g (12.7 mmol) of ca 50% hydrogen peroxide, and 11.7 ml of dichloromethane. The reaction mixture was left to stand at room temperature 90 min, whereupon the same amount of trifluoroperacetic acid as before was added and the mixture was again left to stand for another 1 h. The mixture was then poured into water and the product was extracted with chloroform. The organic layer was separated, washed with 10% solution of potassium hydrogen carbonate and water, and dried. The solvent was distilled off to leave 760 mg of evaporation residue which was purified by column chromatography (200 g silica gel, chloroform–ether 1 : 1). Yield 340 mg (41%) of pure lactone, m.p. 211 – 215 °C (aqueous ethanol). IR spectrum: 3 630, 3 450 (O–H); 1 735 and 1 192 (ester); 1 735, 1 160, 1 090 (lactone); 1 076, 1 031 (C–O). ^{1}H NMR spectrum: 0.66 s, 3 H (3 × H-18); 0.92 s, 3 H (3 × H-19); 0.90 t, 3 H (CH_3CH_2 of the ester group, J = 7.5); 1.14 d, 3 H (3 × H-21, J = 7); 1.11 d, 3 H (CH_3CH of the ester group, J = 7); 3.69 m, 1 H (H-2β, $W_{1/2}$ = 21); 3.86 – 4.29 m, 3 H (H-3β and 2 × H-7a); 4.84 m, 1 H (H-20, $W_{1/2}$ = 17). For $C_{26}H_{42}O_6$ (450.6) calculated: 69.30% C, 9.40% H; found: 69.41% C, 9.08% H.

(20*R*)-Pregn-5-ene-3β,20-diol 3-(4-Methylbenzenesulfonate) 20-(2-Methylbutyrate) (*XI*)

A solution of 700 mg (1.48 mmol) of (20*R*)-pregn-5-ene-3,20-diol 3-(4-methylbenzenesulfonate) (*X*) in 50 ml of pyridine was treated with a fresh solution of 2 g (1.65 mmol) of 2-methylbutanoyl chloride in 4 ml of pyridine. The mixture was left to stand at room temperature 18 h, whereupon it was submitted to extraction with ether in usual way. As the compound could not be obtained in crystalline form and decomposed during attempts at its purification, it was used for further purposes in the form of the oily evaporation residue which was obtained by working up the reaction mixture.

(20*R*)-3α,5α-Cyclopregnane-6β,20-diol 20-(2-Methylbutyrate) (*XII*)

The raw tosylate *XI* (1.04 g, max. 1.87 mmol) was dissolved in 30 ml of acetone, and 9.5 ml of water and 2 g (23 mmol) of potassium carbonate were added thereto. The mixture was refluxed 150 min, cooled, poured into water, and the product was extracted with chloroform. The organic layer was separated, washed with water, dried with anhydrous sodium sulfate, and the solvent was distilled off to give 839 mg of evaporation residue. The product was purified by column chromatography (100 g silica gel, petroleum ether–ether 10 : 1) to give 620 mg (96%) of oily rearranged product *XII*. IR spectrum: 3 625 (O–H); 3 070 (cyclopropane); 1 730, 1 193 (COOR); 1 076 (C–O). ^{1}H NMR spectrum: 0.19 – 0.39 and 0.39 – 0.60 m, 2 × 1 H (2 × proton of cyclopropane ring); 0.69 s, 3 H (3 × H-18); 0.90 t, 3 H (CH_3CH_2 of the ester group, J = 7); 1.05 s, 3 H (3 × H-19); 1.11 d, 3 H (CH_3CH of the ester group, J = 7); 1.13 d, 3 H (3 × H-21, J = 6.5); 2.09 – 2.54 m, 1 H (CH_3CH of the ester group); 3.26 m, 1 H (H-6α, $W_{1/2}$ = 5); 4.84 m, 1 H (H-20, $W_{1/2}$ = 18). Mass spectrum (*m/z*): 402 (M^+). For $C_{26}H_{42}O_3$ (402.6) calculated: 77.56% C, 10.52% H; found: 77.72% C, 10.60% H.

(20*R*)-20-Hydroxy-3α,5-cyclo-5α-pregnan-6-one 20-(2-Methylbutyrate) (*XIII*)

The Jones reagent was added dropwise to a solution of 80 mg (0.2 mmol) of 6β-alcohol *XII* in 2 ml of acetone until permanent brown colour. After 10 min standing, 0.5 ml of methanol was added, and after another 5 min standing the mixture was poured into water and worked up by extraction with ether in the usual way. Yield 72 mg (90%) of ketone *XIII*, m.p. 78 – 79 °C. IR spectrum: 3 030 (cyclopropane); 1 731, 1 192 (COOR); 1 697 (C=O, ketone). ^{1}H NMR spectrum: 0.69 s, 3 H (3 × H-18); 0.92 t, 3 H (CH_3CH_2 of the ester group, J = 7); 1.01 s, 3 H (3 × H-19); 1.15 d, 3 H (CH_3CH of the ester group, J = 7); 1.16 d, 3 H (3 × H-21, J = 6.5); 2.17 – 2.56 m, 2 H (H-7); 4.85 m, 1 H (H-20, $W_{1/2}$ = 19). For $C_{26}H_{40}O_3$ (400.6) calculated: 77.95% C, 10.07% H; found: 77.95% C, 10.19% H.

(20*R*)-20-Hydroxy-5α-pregn-2-en-6-one 20-(2-Methylbutyrate) (*XIV*)

A solution of 0.51 g (1.3 mmol) of 3α,5α-cyclocompound *XIII* in 5 ml of N,N-dimethylacetamide was treated with 47.5 mg (0.19 mmol) of pyridinium 4-methylbenzenesulfonate[5] and 54 mg (0.62 mmol) of lithium bromide. The mixture was heated under nitrogen at 160 °C 6 h. After cooling, the mixture was poured into water, the product was extracted with ether, and the ethereal layer was worked-up in the usual way. The evaporation residue (570 mg) was purified by column chromatography (100 g silica gel, petroleum ether–ether 9 : 1). The work-up of the respective fractions gave 370 mg (73%) of olefin *XIV*, m.p. 118 – 120 °C (aqueous methanol). IR spectrum: 3 030, 1 659 (double bond); 1 719, 1 191 (COOR); 1 710 inflection (C=O, ketone). ^{1}H NMR spectrum: 0.65 s, 3 H and 0.71 s, 3 H (3 × H-18 and 3 × H-19); 0.90 t, 3 H (CH_3CH_2 of the ester group, J = 7); 1.15 d, 6 H (3 × H-21 and CH_3CH of the ester group, J = 7); 4.81 m, 1 H (H-20, $W_{1/2}$ = 17); 5.58 m, 2 H (H-2 and H-3, $W_{1/2}$ = 9). For $C_{26}H_{40}O_3$ (400.6) calculated: 72.95% C, 10.07% H; found: 78.15% C, 10.00% H.

REFERENCES

1. Kohout L.: Collect. Czech. Chem. Commun. *54*, 3348 (1989).
2. Kohout L., Velgová H., Strnad M., Kamínek M.: Collect. Czech. Chem. Commun. *52*, 476 (1987).
3. Velgová H., Lábler L., Černý V., Šorm F., Sláma K.: Collect. Czech. Chem. Commun. *33*, 242 (1968).
4. Mitchell J. W., Livingstone G. A.: *Methods of Studying Plant Hormones and Growth Regulating Substances*, p. 26, Agricultural Handbook No. 336. U.S. Government Printing Office, Washington, D.C. 1968.
5. Watanabe T., Kuriyama H., Furuse T., Kobayashi K., Takatsuto S.: Agric. Biol. Chem. *52*, 2117 (1988).

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