2α,3α-DIHYDROXY-7-OXA-6-OXO-B-HOMO-5α-PREGNAN-21-OIC ACID AND ITS DERIVATIVES AS BRASSINOLIDE ANALOGUES*

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The synthesis of compounds *VIIIb,c,f,h* and their plant growth-promoting activity is reported. These compounds differ from the previously described substances *Ia—d* by the absence of the 21-methyl group, this structural change resulting in a dramatic decrease in the growth-promoting activity.

In our previous paper[1] we prepared 2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanic acid (*Ia*) and some of its esters and amides (*e.g. Ib—d*). This acid showed relatively high brassinolide activity; the activity (though lower) was also found for its ester and amide derivatives. These results offered the possibility to establish the importance of the 21-methyl group by comparing the activities of the acid *Ia* and its congeners with those of the analogues lacking the 21-methyl group since such analogues could be readily accessible.

The syntheses and biological assays of such compounds are described in the present paper. For the preparation of the above substances we set out from the known methyl ester *IIIa*. The synthesis of this substance from 3β-hydroxy-5-androsten-17-one was described by Plattner and Schreck[2]. We prepared the ester *IIIa* from 3β-acetoxy-5-etienic acid (*II*) using the Arndt–Eistert reaction followed by partial hydrolysis (*IIIb* → *IIIa*). Tosylation of *IIIa*, solvolysis of the tosylate *IIIc* to the corresponding 3α,5α-cyclo-6β-hydroxysteroid followed by Jones' oxidation was accomplished without purification of the intermediates and gave the 3α,5α-cyclo-6-oxosteroid *IV* in good yield. The latter compound reacted with hydrogen bromide in acetic acid in a rapid and smooth reaction to give the 3β-bromo derivative *V* which on treatment with LiBr–Li_2CO_3 in boiling dimethylformamide gave the 2,3-unsaturated compound *VI*. Catalytic osmylation of *VI* yielded the diol *VIIa*, which on acetylation followed by treatment of the diacetate *VIIb* with trifluoro-peroxyacetic acid furnished a mixture of two lactones which were separated by chromatography. The major component (68%) has the desired structure *VIIIa* as

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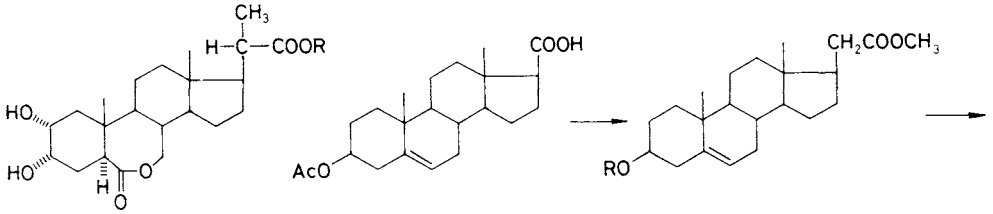
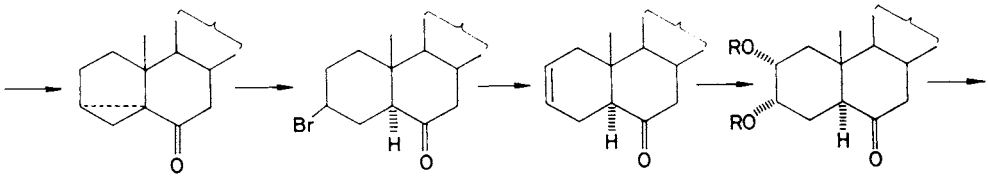
proven by its ^{1}H NMR spectrum. The regioisomeric lactone *IX* was isolated in about 12% yield. The osmylation of the 2,3-double bond is not completely stereoselective since some 2β,3β-diacetoxy derivative *X* could be isolated when the mother liquors after crystallization of the diol *VIIa* were acetylated and oxidized with trifluoroperoxyacetic acid. A similar observation has already been published[3]. Further



Ia, R = H
Ib, R = CH_3
Ic, R = $NHCH(CH_3)_2$
Id, R = $NHCH_2CH(CH_3)_2$

II

IIIa, R = H
IIIb, R = Ac
IIIc, R = Ts



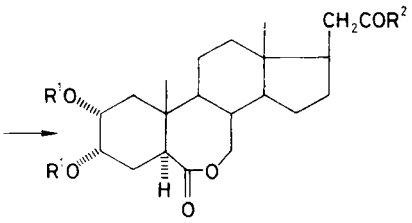
IV *V* *VI*

VIIa, R = H
VIIb, R = Ac

steps involved alkaline hydrolsis of *VIIIa* to the acid *VIIIc*, protection of its hydroxyl groups by acetylation (*VIIId*) and preparation of the corresponding acyl chloride by treatment of *VIIId* with oxalyl chloride. Treatment of the chloride with isopropyl- and isobutylamine yielded the amides *VIIIe* and *VIIIg* which were converted to the desired products *VIIIf* and *VIIIh* by mild alkaline hydrolysis with potassium hydrogen carbonate. The methyl ester *VIIIb* was prepared from *VIIIc* by treatment with diazomethane.

Biological assays (Table I) show that the compounds lacking the 21-methyl group (*VIIIb,c,f,h*) are less active than the corresponding compounds with normal structure (*Ia–d*)[1]. The decrease of activity is dramatic (by about two orders) when the methyl group is removed in compounds with higher activity (*Ia,c vs VIIIc,f*) but is less pronounced when this structural change is performed on less active substances (by

ca one order for *Id vs VIIIh* and roughly about 50% for *Ib vs VIIIb*). These results show the importance of the 21-methyl for the growth-promoting activity.



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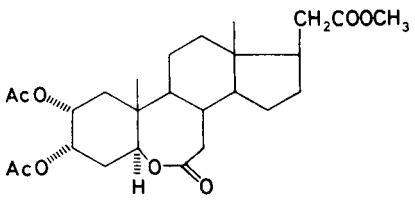
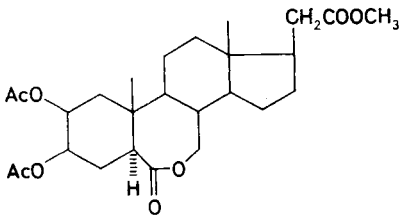


IX

VIII a, R^1 = Ac; R^2 = OCH_3
VIII b, R^1 = H ; R^2 = OCH_3
VIII c, R^1 = H ; R^2 = OH
VIII d, R^1 = Ac; R^2 = OH
VIII e, R^1 = Ac; R^2 = $NHCH(CH_3)_2$
VIII f, R^1 = H ; R^2 = $NHCH(CH_3)_2$
VIII g, R^1 = Ac; R^2 = $NHCH_2CH(CH_3)_2$
VIII h, R^1 = H ; R^2 = $NHCH_2CH(CH_3)_2$



X

TABLE I

Biological activities of brassinosteroids in the bean second internode elongation assay

	Elongation (mm) at quantities (mol)						Quantity required for maximum elongation (mol)[a]
	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}	
24-epibrassinolide	18·5	31·0	32·8	20·4	12·2	8·2	$1{\cdot}0 . 10^{-10}$
Ia[1]	9·5	13·2	20·8	12·8	6·7	0·7	$1{\cdot}0 . 10^{-10}$ ($1{\cdot}4 . 10^{-11}$)
Ib[1]							$1{\cdot}0 . 10^{-9}$ ($1{\cdot}4 . 10^{-13}$)
Ic[1]	10·4	8·8	12·4	7·8	4·6	0	$1{\cdot}0 . 10^{-10}$ ($1{\cdot}0 . 10^{-12}$)
Id[1]	8·0	5·2	4·8	6·6	4·0	1·8	$1{\cdot}0 . 10^{-11}$ ($7{\cdot}6 . 10^{-14}$)
VIIIb	4·6	8·2	4·1	1·3	0	0	$1{\cdot}0 . 10^{-9}$ ($3{\cdot}1 . 10^{-13}$)
VIIIc	4·2	7·3	8·1	7·6	4·2	0·5	$1{\cdot}0 . 10^{-10}$ ($2{\cdot}2 . 10^{-13}$)
VIIIf	10·5	7·8	6·5	7·4	3·6	2·1	$1{\cdot}0 . 10^{-11}$ ($8{\cdot}2 . 10^{-14}$)
VIIIh	5·4	3·9	5·3	3·6	0	0	$1{\cdot}0 . 10^{-10}$ ($5{\cdot}0 . 10^{-13}$)

[a] In parentheses: Quantity of 24-epibrassinolide causing the same maximum elongation (first peak) as the tested substance.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotation measurements were carried out in chloroform with an error of ±3°. The infrared spectra were recorded on a Zeiss UR 20 or on a Perkin–Elmer spectrometer. The ^{1}H NMR spectra were measured on a Varian XL 200 instrument, in deuteriochloroform with tetramethylsilane as internal reference.

The bean second internode bioassay sensitized by Strnad and co-workers[4]: Bean seeds (*Phaseolus vulgaris* L., *cv* PINTO) were germinated for two days and selected germinating seeds were transferred into pots containing perlite and grown[5] 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 48 W/m^2, light-dark period 16/8 h). The tested compounds were applied in fractionated lanoline as 2 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 are 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.

Methyl 3β-Acetoxy-5-pregnen-21-oate (*IIIb*)

3β-Acetoxy-5-etienic acid[6] *II* (2 g) was dissolved in benzene (150 ml) and the solution concentrated to 120 ml. After cooling to 33°C, oxalyl chloride (4 ml) was added and let stand for 4·5 h at room temperature. The solvent and excess reagent were removed *in vacuo* at <33°C and evaporated twice with benzene (30 ml each portion). The residue was dissolved in benzene (20 ml) and this solution was added dropwise to a stirred solution (5°C) of diazomethane (prepared from 10 g of N-nitroso-N-methylurea with 40% aqueous KOH using ether (100 ml) dried over sodium. The solution of diazomethane was distilled and dried with KOH for 2 h). After stirring for 30 min, the mixture was concentrated in vacuo at room temperature to a volume of several ml, methanol was added with stirring and the mixture heated to 50°C. A slurry of Ag_2O in methanol (10 ml) was prepared from the stirred aqueous $AgNO_3$ (10% 4 ml) to which excess aqueous NaOH (10%) was slowly added, decanted repeatedly with distilled water, the precipitate collected on a Büchner funnel, washed thoroughly with water and suspended in MeOH. Half of this slurry was added to the vigorously stirred solution of diazoketone heated to 50–60°C for 15 min. The rest of the slurry was added with continuous stirring in 6 portions and at 5 min intervals at 60°C. The mixture was then refluxed for 15 min, some active coal added, refluxed shortly and the adsorbent filtered off; the filtrate was concentrated to give crystals which after recrystallization from methanol gave the product (1·21 g), m.p. 129–130°C, $[\alpha]_D$ −63° (c = 1·6). Literature[2] reports m.p. 128–129°C, $[\alpha]_D$ −57° ($CHCl_3$). IR spectrum (CCl_4): 1 741, 1 733 cm^{-1} ($OCOCH_3$, $COOCH_3$); 1 249 cm^{-1} ($OCOCH_3$); 1 166, 1 438 cm^{-1} ($COOCH_3$); 1 671, 3 030 cm^{-1} (double bond).

Methyl 3β-Hydroxy-5-pregnen-21-oate (*IIIa*)

The acetoxy derivative *IIIb* (25 g) was dissolved in $CHCl_3$ (60 ml), CH_3OH (1 250 ml) and hydrochloric acid (36%, 25 ml) were added and the mixture kept at room temperature for 24 h. $NaHCO_3$ was then added and the mixture concentrated *in vacuo*. After the addition of water the product was taken up in ether, the solution washed with water several times, then with $NaHCO_3$ and water. After drying over $MgSO_4$ and evaporation, the residue (23 g) was dissolved in ether to give the product which after crystallization from ether gave the pure compound *IIIa*

(19·2 g), m.p. 131—133°C, $[\alpha]_D$ −65° (dioxan), c = 1·3); literature[2] reports m.p. 128—129°C, $[\alpha]_D$ −63° (dioxan).

Methyl 6-Oxo-3α,5-cyclo-5α-pregnan-21-oate (*IV*)

The methyl ester *IIIa* (1 g) was treated with *p*-toluenesulfonyl chloride (1·5 g) in pyridine (20 ml) for two days at room temperature. The mixture was poured onto ice, diluted with water, the product collected by suction and washed with water. This product (checked by TLC) was added with stirring to a boiling solution of potassium acetate (0·8 g) in water (30 ml), acetone (125 ml) and pyridine (4 ml). The reaction mixture was refluxed and stirred for 1 h, then let stand at room temperature overnight. After this time, the conversion was complete (TLC). Most of the solvent was removed at max. 45°C *in vacuo*, the mixture poured onto ice and extracted with ether. After repeated washing with water and drying with $MgSO_4$, the solvent was removed under reduced pressure and the residue was dissolved in acetone (50 ml), cooled to 5°C and Jones' reagent (3 ml, 17·7% CrO_3) was added at once. After standing in an ice bath for 15 min, excess reagent was decomposed with methanol and water was added until incipient crystallization. After 1 h, the crystals were collected by suction, washed with water and chromatographed on silica gel in petroleum ether–benzene (4 : 1) to yield the product *IV* (700 mg). Crystallization from aqueous acetone furnished the analytical sample, m.p. 96—98°C, $[\alpha]_D$ +33° (c = 1·6). IR spectrum (CCl_4): 1 742, 1 737, 1 161 cm^{-1} ($COOCH_3$); 1 692 cm^{-1} (carbonyl conj. with cyclopropane); 3 080 (3 025, 3 005) cm^{-1} (cyclopropane ring). For $C_{22}H_{32}O_3$ (344·5) calculated: 76·70% C, 9·36% H; found: 76·88% C, 9·42% H.

Methyl 3β-Bromo-6-oxo-5α-pregnan-21-oate (*V*)

The 3α,5α-cyclosteroid *IV* (200 mg) was dissolved in acetic acid (1 ml) and a solution of hydrogen bromide in acetic acid (0·6 ml, 35% HBr) was added. After standing at room temperature for 15 min, the reaction was complete (TLC). Crystallization from aqueous acetone yielded the pure product V (175 mg), m.p. 149·5—151·5°C, $[\alpha]_D$ −12° (c = 1·2). For $C_{22}H_{33}BrO_3$ (425·4) calculated: 62·11% C, 7·82% H, 18·78% Br; found: 62·36% C, 7·69% H, 19·21% Br.

Methyl 6-Oxo-5α-pregn-2-en-21-oate (*VI*)

The compound *V* (450 mg), LiBr (700 mg), Li_2CO_3 (80 mg) and dimethylformamide (10 ml, distilled over P_2O_5) were refluxed under argon for 50 min. The mixture was cooled, poured into water and extracted with ether. The solution was washed with water, HCl, $NaHCO_3$ and water, dried with $MgSO_4$ and the solvent removed *in vacuo*. TLC showed the presence of a more polar compound with R_F idenical to that of 3α,5α-6-oxosteroid. Chromatography on silica gel (10 g) in petroleum ether–benzene (8 : 2) separated the desired compound (200 mg); benzene–ether (2%) eluted the more polar impurity (60 mg). Crystallization of the major component from aqueous acetone yielded the product (165 mg), m.p. 123—125°C, $[\alpha]_D$ +24° (c = 1·6). For $C_{22}H_{32}O_3$ (344·5) calculated: 76·70% C, 9·36% H; found: 77·11% C, 9·36% H.

Methyl 2α,3α-Dihydroxy-6-oxo-5α-pregnan-21-oate (*VIIa*)

Water (15 ml), N-methylmorpholine N-oxide (2·1 g) and osmium tetroxide (100 mg) dissolved in tert-butyl alcohol (c. 5 ml) were successively added to the unsaturated ester *VI* (1·4 g), dissolved in tetrahydrofuran (75 ml). The mixture was stirred under argon for 6 h at room temperature, let stand overnight, then $Na_2SO_3.7\,H_2O$ (6 g) in water (30 ml) was added and the mixture stirred for 4 h. After dilution with water the product was taken up in dichloromethane, washed with 5% HCl, $NaHCO_3$, and water, the solvent evaporated and the residue (1·62 g) crystallized

from aqueous methanol, dissolved in chloroform and precipitated by the addition of heptane. The precipitation was repeated twice and the product crystallized from methanol to give the diol *VIIa* (714 mg), m.p. 164–165°C, $[\alpha]_D$ −7° (c = 1·5). IR spectrum ($CHCl_3$): 3 620, 3 570, 3 500, 1 048 cm^{-1} (OH); 1 748, 1 440, 1 162 cm^{-1} ($COOCH_3$); 1 712 cm^{-1} (CO). For $C_{22}H_{34}O_5$ (378·5) calculated: 69·81% C, 9·05% H; found: 69·70% C, 9·10% H.

Methyl 2α,3α-Diacetoxy-6-oxo-5α-pregnan-21-oate (*VIIb*)

The diol *VIIa* (631 mg) was acetylated in pyridine (10 ml) with acetic anhydride (3 ml) at room temperature overnight, the mixture was then heated at 65°C for 3 h and worked up in the usual manner to give the product (716 mg) which after crystallization from methanol amounted to 632 mg, m.p. 198·5–199°C, $[\alpha]_D$ −20° (c = 1·4). IR spectrum ($CHCl_3$): 1 739, 1 260, 1 044 cm^{-1} ($OCOCH_3$); 1 726, 1 158, 1 440 cm^{-1} ($COOCH_3$); 1 712 cm^{-1} (CO). IR spectrum (CCl_4): 1 750, 1 250, 1 232, 1 046 cm^{-1} ($OCOCH_3$); 1 742, 1 439, 1 157 cm^{-1} ($COOCH_3$); 1 718 (CO). For $C_{26}H_{38}O_7$ (462·6) calculated: 67·51% C, 8·28% H; found: 67·28% C, 7·96% H.

Methyl 2α,3α-Diacetoxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-oate (*VIIIa*)

A solution of trifluoroperoxyacetic acid prepared from trifluoroacetic acid anhydride (10·7 ml) and H_2O_2 (79%, 1·87 ml) in dichloromethane (150 ml) at 0°C was added to a solution of the ketone *VIIb* (4·77 g) in dichloromethane (60 ml). The mixture was stirred several minutes at 0°C, then at room temperature. After 4 h the mixture was washed with water, $NaHCO_3$ and water, dried and evaporated. Repeated chromatography on silica gel (120 g) in benzene and benzene–ether (2%) separated the major product (3·4 g) and the more polar minor component (399 mg). The major product was crystallized from ether to give the diacetate *VIIIa* (3·31 g), m.p. 157–158·5°C, $[\alpha]_D$ +40° (c = 1·3). IR spectrum ($CHCl_3$): 1 738 cm^{-1} ($OCOCH_3$, $COOCH_3$); 1 256, 1 050 cm^{-1} ($OCOCH_3$); 1 729, 1 078 cm^{-1} (lactone); 1 440 cm^{-1} ($COOCH_3$); 1 176 (lactone, $COOCH_3$). ^1H NMR spectrum: 0·64 s, 3 H (H-18); 0·99 s, 3 H (H-19); 2·00 s, 3 H and 2·12 s, 3 H (2 × $OCOCH_3$); 3·66 s, 3 H ($COOCH_3$); 2·30 ddd, 1 H (H-4β, $J_{4\beta,4\alpha}$ = = −16·0; $J_{4\beta,5}$ = 12·4, $J_{4\beta,3}$ = 2·4); 3·01 dd, 1 H (H-5, $J_{5,4\beta}$ = 12·4, $J_{5,4\alpha}$ = 4·8); 4·02 to 4·17 m, 2 H (H-7); 4·87 ddd, 1 H (H-2, $J_{2,1\alpha}$ = 12·6, $J_{2,1\beta}$ = 4·8, $J_{2,3}$ = 2·7); 5·37 m, 1 H (H-3, $\sum J$ = 9·0). For $C_{26}H_{38}O_8$ (478·6) calculated: 65·24% C, 8·00% H; found: 65·33% C, 7·80% H.

Methyl 2α,3α-Dihydroxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-oate (*VIIIb*)

The acid *VIIIc* (340 mg) was suspended in ether (10 ml) and methanol (10 ml), treated with an ethereal solution of diazomethane and the solution concentrated to a small volume to provide the ester *VIIIb* (310 mg), m.p. 216–218°C, $[\alpha]_D$ +39° (c = 1·2). IR spectrum ($CHCl_3$): 3 610, 3 580 cm^{-1} (OH); 1 725 ($COOCH_3$, lactone); 1 439, 1 175 cm^{-1} ($COOCH_3$); 1 320 cm^{-1} (lactone). For $C_{22}H_{34}O_6$ (394·5) calculated: 66·98% C, 8·69% H; found: 67·31% C, 8·85% H.

2α,3α-Dihydroxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-oic Acid (*VIIIc*)

A mixture of the ester *VIIIa* (3·2 g), aqueous NaOH (36%, 20 ml) and ethanol (100 ml) was refluxed for 18 h, cooled, acidified with hydrochloric acid, concentrated to one half of the original volume and diluted with water (400 ml). The separated crystals (2·53 g) were collected on a Büchner funnel and crystallized from chloroform–benzene to give the acid (2·11 g), m.p. 290–294°C. Crystallization from aqueous ethanol raised the m.p. to 294–295°C. IR spectrum (KBr): 3 465, 3 405, 1 070, 1 032 cm^{-1} (OH); 3 200–2 400, 1 709, 1 695 cm^{-1} (COOH); 1 729, 1 228, 1 199,

1 186 cm^{-1} (lactone). For $C_{21}H_{32}O_6$ (380·5) calculated: 66·29% C, 8·48% H; found: 66·49% C, 8·50% H.

2α,3α-Diacetoxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-oic Acid (*VIIId*)

The acid *VIIIc* (2·1 g) was acetylated in pyridine (80 ml) with acetic acid anhydride (40 ml) at room temperature for 18 h. Water (35 ml) was then added and heated at 90–92°C for 1 h. Addition of ice and acidification with hydrochloric acid led to crystallization of the product which was collected by suction after 2 h. Crystallization from aqueous acetic acid and then from aqueous acetone gave pure acid (2·03 g), m.p. 245–247°C, $[\alpha]_D$ +48° (c = 1·2). IR spectrum ($CHCl_3$): 3 400–2 400, 1 710 cm^{-1} (COOH); 1 740, 1 254 cm^{-1} ($OCOCH_3$); 1 729 sh, 1 179 cm^{-1} (lactone). For $C_{25}H_{36}O_8$ (464·5) calculated: 64·63% C, 7·81% H; found: 64·84% C, 8·01% H.

N-Isopropyl 2α,3α-Diacetoxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-amide (*VIIIe*)

The acid *VIIId* (800 mg) was dissolved in benzene (150 ml) and this solution was concentrated to about 75 ml; oxalyl chloride (2 ml) was added, the mixture was let stand for 5 h and evaporated (at max. 35°C). Traces of oxalyl chloride were removed by repeated addition of dry benzene and its removal in vacuo (max. 35°C). The residue was dissolved in benzene (40 ml). One half of this solution (20 ml) was treated with isopropylamine (1 ml) at room temperature overnight, diluted with benzene, washed with water, 5% HCl, water, $NaHCO_3$ and dried with $MgSO_4$. Evaporation of the solvent provided the crystalline amide (530 mg) which after crystallization from aqueous acetone gave the product (302 mg, m.p. 139–142°C, $[\alpha]_D$ +37° (c = 1·2). IR spectrum ($CHCl_3$): 1 738, 1 257 cm^{-1} ($OCOCH_3$); 1 728 sh, 1 179 cm^{-1} (lactone); 3 440, 1 661, 1 519 cm^{-1} (CONH). For $C_{28}H_{43}NO_7.H_2O$ (523·7) calculated: 64·22% C, 8·66% H, 2·68% N; found: 64·03% C, 8·39% H, 2·65% N.

N-Isopropyl 2α,3α-Dihydroxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-amide (*VIIIf*)

A solution of $KHCO_3$ (200 mg) in water (1·5 ml) was added to the diacetate *VIIIe* (230 mg) dissolved in methanol (45 ml) and the mixture was refluxed for 40 min. The solution was concentrated to a small volume, water was added and the product taken up in chloroform. The residue was an amorphous solid, $[\alpha]_D$ +46° (c = 1·8), IR spectrum ($CHCl_3$): 3 620, 3 580 cm^{-1} (OH); 3 440, 1 666, 1 556, 1 539 cm^{-1} (CONH); 1 729 sh, 1 176 cm^{-1} (OCO). For $C_{24}H_{39}NO_5$ (421·6) calculated: 68·37% C, 9·32% H, 3·32% N; found: 68·74% C, 9·24% H, 3·14% N.

N-Isobutyl 2α,3α-Diacetoxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-amide (*VIIIg*)

The second half (20 ml) of the acid chloride solution (as described under *VIIIe* was treated with isobutylamine (1 ml) in the same manner as in the case of the isopropyl derivative. The product was obtained as oil (457 mg) which crystallized after standing. Crystallization from benzene–ether gave the pure amide *VIIIg*, m.p. 123–124°C, $[\alpha]_D$ +33° (c = 1·1). IR spectrum (CCl_4): 1 739, 1 256 cm^{-1} ($OCOCH_3$); 1 729 sh, 1 177, 1 076 cm^{-1} (lactone); 3 455, 1 664, 1 524 cm^{-1} (CONH). For $C_{29}H_{45}NO_7$ (519·7) calculated: 67·02% C, 8·73% H, 2·69% N; found: 67·05% C, 8·64% H, 2·54% N.

N-Isobutyl 2α,3α-Dihydroxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-amide (*VIIIh*)

A solution of $KHCO_3$ (220 mg) in water (1·5 ml) was added to the diacetate *VIIIg* (253 mg) in methanol (52 ml) and the mixture was refluxed for 40 min. The solution was concentrated to

a small volume, diluted with water and the product taken up in chloroform. The residue was an amorphous solid (190 mg), $[\alpha]_D$ +43° (c = 1·5). IR spectrum ($CHCl_3$): 3 610, 3 570, 1 073, 1 029 cm^{-1} (OH); 3 450, 1 661, 1 530 cm^{-1} (CONH); 1 721, 1 178 cm^{-1} (lactone). For $C_{25}H_{41}NO_5$ (435·6) calculated: 68·92% C, 9·49% H, 3·22% N; found: 69·16% C, 9·47% H, 3·43% N.

Methyl 2 α,3α-Diacetoxy-6-oxa-7-oxo-B-homo-5α-pregnan-21-oate (*IX*)

The minor product from the treatment of the ketone *VIIb* with trifluoroperoxaacetic acid was crystallized from methanol to give *IX* (257 mg), m.p. 244—246°C, $[\alpha]_D$ +8° (c = 1·9). IR spectrum ($CHCl_3$): 1 740 cm^{-1} ($OCOCH_3$, $COOCH_3$); 1 256, 1 050 cm^{-1} ($OCOCH_3$); 1 439, 1 160 cm^{-1} ($COOCH_3$); 1 727, 1 030, 1 072 cm^{-1} (lactone). ^{1}H NMR spectrum: 0·63 s, 3 H (H-18); 1·01 s, 3 H (H-19); 1·99 s, 3 H and 2·12 s, 3 H (2 × $OCOCH_3$); 2·45—2·59 m, 2 H (H-7); 3·66 s, 3 H ($COOCH_3$); 4·49 dd, 1 H (H-5, $J_{5,4\beta}$ = 10·8, $J_{5,4\alpha}$ = 6·0); 4·90 ddd, 1 H (H-2, $J_{2,1\alpha}$ = 12·4, $J_{2,1\beta}$ = 4·7, $J_{2,3}$ = 2·7); 5·38 m, 1 H (H-3, $\sum J$ = 10·7). For $C_{26}H_{38}O_8$ (478·6) calculated: 65·24% C, 8·00% H; found: 65·53% C, 8·28% H.

Methyl 2β,3β-Diacetoxy-7-oxa-6-oxo-B-homo-5α-pregnan-21-oate (*X*)

The mother liquors (4 g) after crystallization of several batches of the diol *VIIa* were acetylated and the crude diacetate was treated with trifluoroperoxyacetic acid as described above. After the usual workup the acetylated material was crystallized from methanol and then chromatographed on silica gel (50 g). The major fraction (530 mg), after crystallization from methanol, gave the diacetate *XI* (440 mg, m.p. 295—297°C, $[\alpha]_D$ +46° (c = 1·5). IR spectrum ($CHCl_3$): 1 736, 1 729 cm^{-1} ($OCOCH_3$, $COOCH_3$, lactone); 1 259, 1 050 cm^{-1} ($OCOCH_3$); 1 439, 1 170 cm^{-1} ($COOCH_3$); 1 059, 1 021 cm^{-1} (lactome). ^{1}H NMR spectrum: 0·63 s, 3 H (H-18); 1·05 s, 3 H (H-19); 2·02 s, 3 H and 2·09 s, 3 H (2 × $OCOCH_3$); 3·66 s, 3 H ($COOCH_3$); 2·31 dd, 1 H (H-1β, $J_{1\beta,1\alpha}$ = −15·2, $J_{1\beta,2}$ = 3·8); 2·53 ddd, 1 H (H-4β, $J_{4\beta,4\alpha}$ = −13·6, $J_{4\beta,3}$ = 12·9, $J_{4\beta,5}$ = 12·4); 3·01 dd, 1 H (H-5, $J_{5,4\beta}$ = 12·4, $J_{5,4\alpha}$ = 4·2); 4·03 dd, 1 H (H-7α, $J_{7\alpha,7\beta}$ = −12·6, $J_{7\alpha,8}$ = 8·3); 4·13 dd, 1 H (H-7β, $J_{7\beta,7\alpha}$ = −12·6, $J_{7\beta,8}$ = 2·6); 4·80 ddd, 1 H (H-3, $J_{3,2}$ = 2·8, $J_{3,4\alpha}$ = 4·2, $J_{3,4\beta}$ = 12·9); 5·24 m, 1 H (H-2, $\sum J$ = 11·0). For $C_{26}H_{38}O_8$ (478·6) calculated: 65·24% C, 8·00% H; found: 65·60% C, 7·91% H.

The elemental analyses were carried out in the Analytical Laboratory of this Institute under the direction of Dr J. Horáček. The infrared spectra were recorded by Mrs K. Matoušková and interpreted by Dr J. Smolíková.

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