PREGNANE-TYPE BRASSINOSTEROIDS WITH A FOUR-CARBON ESTER FUNCTIONALITY IN POSITION 20*

Ladislav KOHOUT[a1], Alexander KASAL[a2] and Miroslav STRNAD[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] kohout@uochb.cas.cz, [2] kasal@marylin.uochb.cas.cz*

[b] *Institute of Experimental Botany, Academy of Sciences of the Czech Republic, 772 00 Olomouc, Czech Republic*

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Analogues of brassinolide and castasterone, containing an ester functionality with four carbon atoms in the position 20 of the pregnane skeleton, have been prepared. In the bean second internode assay the most effective compounds were (20*R*)-2α,3α,20-trihydroxy-5α-pregnan-6-one 20-isobutyrate (**6d**) and (20*R*)-2α,3α,20-trihydroxy-B-homo-7-oxa-5α-pregnan-6-one 20-isobutyrate (**7d**). On the other hand, (20*R*)-2α,3α,20-trihydroxy-B-homo-6-oxa-5α-pregnan-7-one 20-heptafluorobutyrate (**8**) was the first compound with the retarding effect in the same test.

Key words: Brassinosteroids; PGH; Synthesis; Antibrassinolide activity.

Recent studies on brassinosteroids have focused attention on the relationship between molecular structure and biological activity[2]. In one of our previous communications on this topic[3] we reported androstane derivatives of brassinolide with ester functionalities in the position 17. Some of these compounds exhibited high brassinolide activity in the bean second internode assay[4]. Recently, some other brassinosteroid esters have been described[5] the activity of which is higher than that of the parent compounds. In the present communication we describe some pregnane type derivatives with four-carbon ester functionalities in position 20.

Their synthesis (Scheme 1) started from (20*R*)-pregn-5-ene-3β,20-diol 3-tosylate[6] (**1**) which on reaction with chloride or anhydride of the appropriate acid in pyridine afforded the corresponding esters **2a–2d**. Thus, reaction of compound **1** with heptafluorobutyric anhydride gave the heptafluorobutyrate **2a**, with succinic anhydride the hemisuccinate **2b**, with butyryl chloride the butyrate **2c**, and with isobutyryl chloride the isobutyrate **2d**. Reaction with potassium acetate converted the obtained esters **2a–2d** into the 3α,5α-cyclo-6-hydroxy derivatives **3a–3d** which on oxidation with Jones rea-

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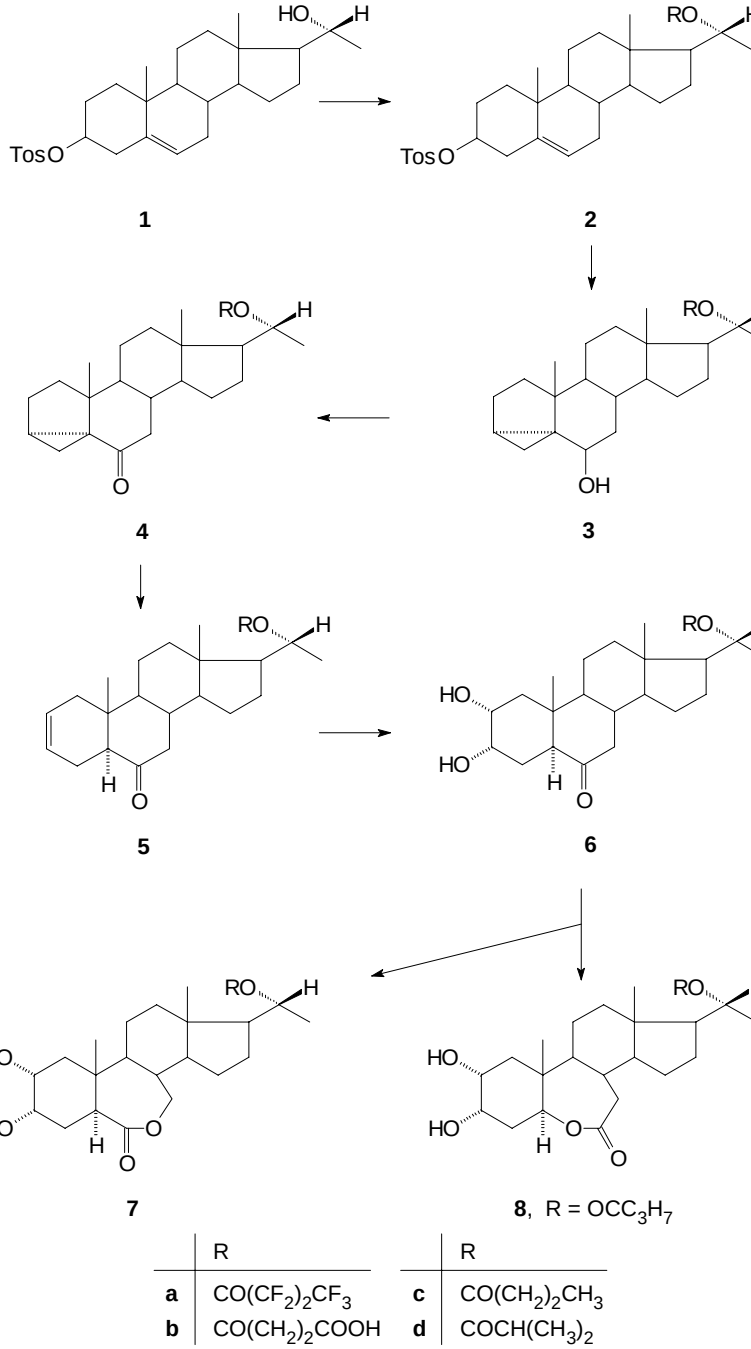

	R		R
a	$CO(CF_2)_2CF_3$	**c**	$CO(CH_2)_2CH_3$
b	$CO(CH_2)_2COOH$	**d**	$COCH(CH_3)_2$

SCHEME 1

gent afforded ketones **4a–4d**. The ketones were treated with lithium bromide in *N*,*N*-dimethylacetamide in the presence of pyridinium tosylate to give 2,3-olefins **5a–5d**. Hydroxylation of these ketones with osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide gave the corresponding castasterone analogues, diols **6a–6d**. The obtained diols **6a–6d** were subjected to the Baeyer–Villiger oxidation with trifluoroperacetic acid in dichloromethane to give the brassinolide analogues, lactones **7a–7d**. The reaction with diol **6a** gave also the isomeric lactone, (20*R*)-2α,3α, 20-trihydroxy-6-oxa-B-homo-5α-pregn-7-one 20-heptafluorobutyrate (**8**). Also in this case we confirmed the known observation[7] that in the Baeyer–Villiger reaction castasterone analogues with 2α,3α-diol grouping give predominantly the 7-oxa lactones of the type **7**, contrary to theoretical expectation.

The biological activity was tested in the bean second internode bioassay[8]. Of all the substances tested, the highest activity was found for compounds **6d** and **7d**; however, even these compounds were less active than 24-epibrassinolide ((22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one).

TABLE I
Bean second internode assays with compounds **5a–5d**, **6a–6d**, **7a–7d** and **8**

Compound	Lengthening of the second internode, mm[a]	Amount applied, mol[b]
24-epiBR[c]	32.3	10^{-10}
5a	6.7	10^{-8}
6a	7.9	10^{-10}
7a	5.6	10^{-7}
8a	–5.4	10^{-10}
5b	2.9	10^{-11}
6b	6.7	10^{-7}
7b	3.6	10^{-7}
5c	9.9	10^{-9}
6c	7.2	10^{-9}
7c	8.6	10^{-8}
5d	5.8	10^{-7}
6d	14.0	10^{-11}
7d	15.1	10^{-9}

[a] Lengthening, compared with that of the reference plant for the amount applied. [b] Amount causing the maximum lengthening of the second internode (all compounds were applied in amounts $1 . 10^{-11}$ to $1 . 10^{-6}$ mol per plant). [c] 24-epiBR: 24-epibrassinolide [(22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one].

To our surprise, the compound **8** retarded substantially the growth at all doses applied, i.e, from $1 . 10^{-11}$ to $1 . 10^{-6}$ mol per plant, the highest retardation being observed with doses $1 . 10^{-7}$ mol per plant. This is the first example of a brassinolide analogue that exhibits antibrassinolide effect.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Bruker IFS 88 spectrometer in tetrachloromethane (unless otherwise stated), wavenumbers are given in cm^{-1}. ^1H NMR spectra were taken in deuteriochloroform on a Varian XL-200 (FT mode, 200 MHz) instrument with tetramethylsilane as internal reference, unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and multiplet half-widths ($W_{1/2}$) in Hz. 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 and proton NMR spectra. Preparative TLC was carried out on 200 × 200 mm plates coated with 0.7 mm thick layer of silica gel Woelm DC. Column chromatography was performed on neutral silica gel 60–120 μm. "Usual work-up" of the solution denotes successive washing with water, 10% HCl, 5% aqueous potassium hydrogen carbonate, water, drying over sodium sulfate, filtration and evaporation of the solvent in vacuo to dryness. Light petroleum was a fraction boiling at 40–62 °C.

(20*R*)-Pregn-5-ene-3β,20-diol 3-Tosylate 20-Heptafluorobutyrate (**2a**)

Hydroxy derivative[6] **1** (841 mg, 1.78 mmol) was dissolved in pyridine (10 ml) and heptafluorobutyric anhydride (945 mg, 2.30 mmol) was added at room temperature. After standing overnight, the reaction mixture was poured into water and the product was taken up in chloroform and worked up as usual. After standing overnight in a refrigerator, the crystalline material was triturated with ether and collected. Crystallization from chloroform–methanol afforded 620 mg (52%) of heptafluorobutyrate **2a**, m.p. 92–96 °C. IR spectrum: 1 779 (ester); 1 379, 1 192, 1 180 (tosylate). ^1H NMR spectrum: 0.64 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 1.27 d, 3 H, J = 6 (3 × H-21); 2.44 s, 3 H (tosylate CH_3); 4.32 m, 1 H, $W_{1/2}$ = 23 (H-3α); 5.06 m, 1 H, $W_{1/2}$ = 19 (H-20); 5.28 d, 1 H, J = 5 (H-6); 7.31 d, 2 H, J = 8 (tosylate H-3 and H-5); 7.80 d, 2 H, J = 8 (tosylate H-2 and H-6). For $C_{32}H_{39}F_7O_5S$ (668.7) calculated: 57.48% C, 5.88% H, 19.89% F, 4.79% S; found: 57.14% C, 6.01% H, 21.50% F, 5.09% S.

(20*R*)-Pregn-5-ene-3β,20-diol 3-Tosylate 20-Hemisuccinate (**2b**)

Triethylamine (300 ml) and succinic anhydride (900 mg, 8.99 mmol) were added to a solution of hydroxy derivative[6] **1** (300 mg, 0.63 mmol) in dichloromethane (18 ml). After standing for 48 h, the mixture was poured into water and worked up in the usual manner. The chloroform solution afforded 115 mg (33%) of noncrystalline title compound. IR spectrum: 3 034 (double bond); 1 733, 1 177 (OCO); 1 716, 942 (COOH); 1 370, 1 189, 1 177 (tosylate). ^1H NMR spectrum: 0.62 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 1.14 d, 3 H, J = 6 (3 × H-21); 2.44 s, 3 H (tosylate CH_3); 4.29 m, 1 H, $W_{1/2}$ = 27 (H-3α); 4.84 m, 1 H, $W_{1/2}$ = 19 (H-20); 5.28 d, 1 H, J = 6 (H-6); 7.30 d, 2 H, J = 8 (tosylate H-3 and H-5); 7.78 d, 2 H, J = 8 (tosylate H-2 and H-6). For $C_{31}H_{44}O_7S$ (560.8) calculated: 66.40% C, 7.91% H, 5.72% S; found: 66.66% C, 8.11% H, 5.55% S.

(20*R*)-Pregn-5-ene-3β,20-diol 3-Tosylate 20-Butyrate (**2c**)

Butyryl chloride (1.45 g, 13.61 mmol) was added at room temperature to a solution of hydroxy derivative[6] **1** (1.25 g, 4.64 mmol) in pyridine (6 ml). After standing overnight, the mixture was poured into water and worked up as usual. The obtained chloroform solution was concentrated and the residue (2.3 g) was purified by chromatography on a column of silica gel (200 g, elution with light petroleum–benzene 1 : 1); yield 0.9 g (63%) of the product **2c**, m.p. 103–104 °C. IR spectrum: 3 070, 3 035, 1 651 (C=C); 1 729, 1 160 (ester); 1 601, 1 445, 1 345, 1 189 (tosylate). ^{1}H NMR spectrum: 0.62 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 1.14 d, 3 H, J = 5.8 (3 × H-21); 0.94 t, 3 H, J = 7.0 (ester CH_3); 2.45 s, 3 H (tosylate CH_3); 4.31 m, 1 H, $W_{1/2}$ = 23 (H-3α); 4.82 m, 1 H, $W_{1/2}$ = 20 (H-20); 5.28 d, 1 H, J = 5 (H-6); 7.32 d, 2 H, J = 8 (tosylate H-3 and H-5); 7.72 d, 2 H, J = 8 (tosylate H-2 and H-6). For $C_{32}H_{46}O_5S$ (542.8) calculated: 70.81% C, 8.54% H, 5.91% S; found: 70.99% C, 8.55% H, 6.11% S.

(20*R*)-Pregn-5-ene-3β,20-diol 3-Tosylate 20-Isobutyrate (**2d**)

Isobutyric anhydride (7.4 g, 52 mmol) was added at room temperature to a solution of hydroxy derivative[6] **1** (7 g, 14.81 mmol) in pyridine (42 ml). After standing overnight, the mixture was poured into water and worked up as usual. The obtained chloroform solution was concentrated and the residue (8.5 g) was chromatographed on a column of silica gel (159 g, elution with light petroleum–ether 9 : 1); yield 7.2 g (89%) of product which was crystalized from aqueous acetone; m.p. 103–108 °C. IR spectrum: 3 070, 3 030, 1 652 (C=C); 1 728, 1 162 (ester); 1 600, 1 448, 1 344, 1 189, (tosylate). ^{1}H NMR spectrum: 0.62 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 1.12 d, 3 H, J = 6.1 (3 × H-21); 1.144 d and 1.151 d, 2 × 3 H, J = 7.9 ((C**H**$_3$)$_2$CHCO); 2.45 s, 3 H (tosylate CH_3); 4.31 m, 1 H, $W_{1/2}$ = 18 (H-3α); 4.82 m, 1 H, $W_{1/2}$ = 18 (H-20); 5.29 bd, 1 H, $J \approx 6$ (H-6); 7.32 d, 2 H, J = 8 (tosylate H-3 and H-5); 7.80 d, 2 H, J = 8 (tosylate H-2 and H-6). For $C_{32}H_{46}O_5S$ (542.8) calculated: 70.81% C, 8.54% H, 5.91% S; found: 70.93% C, 8.54% H, 6.13% S.

(20*R*)-3α,5-Cyclo-5α-pregnane-6β,20-diol 20-Heptafluorobutyrate (**3a**)

Potassium acetate (1.4 g, 14.3 mmol) and water (7 ml) were added to a solution of tosylate **2a** (0.79 g, 1.18 mmol) in acetone (21 ml). After boiling for 4 h, the mixture was cooled, poured into water and extracted with chloroform. The chloroform phase was washed with water and dried over sodium sulfate. The solvent was evaporated and the residue (0.7 g) was chromatographed on a column of silica gel (100 g, gradient elution with light petroleum–ether 83 : 17 to 90 : 20) to give 511 mg (84%) of hydroxy derivative **3a**. IR spectrum: 3 620 (hydroxyl); 3 070 (cyclopropane); 1 777, 1 239 (ester). ^{1}H NMR spectrum: 0.20–0.38 m, 1 H and 0.52 t, 1 H, J = 4.5 (two cyclopropane protons); 0.73 s, 3 H (3 × H-18); 1.08 s, 3 H (3 × H-19); 1.29 d, 3 H, J = 6 (3 × H-21); 3.27 m, 1 H, $W_{1/2}$ = 6 (H-6α); 5.11 m, 1 H, $W_{1/2}$ = 18 (H-20). For $C_{25}H_{33}F_7O_3$ (514.5) calculated: 58.36% C. 6.47% H, 25.85% F; found: 58.40% C, 6.44% H, 23.99% F.

(20*R*)-3α,5-Cyclo-5α-pregnane-6β,20-diol 20-Hemisuccinate (**3b**)

Potassium acetate (1.44 g, 14.67 mmol) and water (7 ml) were added to a solution of tosylate **2b** (0.72 g, 1.28 mmol) in acetone (21.4 ml). After boiling for 4 h, the mixture was cooled, poured into water and extracted with chloroform. The chloroform phase was washed with water and dried over sodium sulfate. The solvent was evaporated and the residue (0.6 g) was chromatographed on a column of silica gel (80 g, gradient elution with light petroleum–ether–acetic acid 70 : 30 : 1 via 60 : 40 : 1 to 60 : 40 : 2) to give 235 mg (44%) of hydroxy derivative **3b**, m.p. 203–205 °C. IR spectrum: 3 615, 1 074 (hydroxyl); 3 059, 3 016 (cyclopropane); 3 400, 1 716, 1 225, 1 216 (carboxyl); 1 732, 1 175

(ester). ^{1}H NMR spectrum: 0.18–0.36 mt, 1 H and 0.44–0.58 m, 1 H (two cyclopropane protons); 0.67 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 1.12 d, 3 H, J = 6 (3 × H-21); 2.58 m, 4 H ($OOCCH_2CH_2COO$); 3.23 m, 1 H, $W_{1/2}$ = 4 (H-6α); 4.84 m, 1 H, $W_{1/2}$ = 24 (H-20). For $C_{25}H_{38}O_5$ (418.6) calculated: 71.74% C, 9.15% H; found: 73.27% C, 9.16% H.

(20*R*)-3α,5-Cyclo-5α-pregnane-6β,20-diol 20-Butyrate (**3c**)

Potassium acetate (0.54 mg, 5.5 mmol) and water (2.6 ml) were added to a solution of tosylate **2c** (270 mg, 0.5 mmol) in acetone (8 ml). After boiling for 6 h, the mixture was cooled, set aside in a refrigerator overnight, poured into water and extracted with ether. The ethereal phase was washed with water and dried over sodium sulfate. The solvent was evaporated and the residue (229 mg) was chromatographed on 8 preparative TLC plates, elution with light petroleum–ether 7 : 3. Yield 185 mg (96%) of oily hydroxy derivative **3c**. IR spectrum: 3 615, 3 500, 970 (hydroxyl); 3 058, 3 016 (cyclopropane); 1 731, 1 187, 1 073 (ester). ^{1}H NMR spectrum: 0.29 dd, 1 H, J = 5, J' = 8.2, and 0.52 t, 1 H, J = 4.2 (two cyclopropane protons); 0.69 s, 3 H (3 × H-18); 0.94 t, 3 H, J = 7.3 (ester CH_3); 1.05 s, 3 H (3 × H-19); 1.14 d, 3 H, J = 6.1 (3 × H-21); 2.24 m, 2 H, $W_{1/2}$ = 14 (ester CH_2–CO); 3.26 t, 1 H, J = 4 (H-6α); 4.86 m, 1 H, $W_{1/2}$ = 16 (H-20). For $C_{25}H_{40}O_3$ (388.6) calculated: 77.27% C, 10.38% H; found: 77.55% C, 10.44% H.

(20*R*)-3α,5-Cyclo-5α-pregnane-6β,20-diol 20-Isobutyrate (**3d**)

Potassium acetate (16.2 g, 16.51 mmol) and water (70 ml) were added to a solution of tosylate **2d** (7 g, 12.90 mmol) in acetone (210 ml). After standing overnight at room temperature, the mixture was refluxed for 1 h, cooled, poured into water and extracted with ether. The ethereal phase was washed with water, dried over sodium sulfate and the solvent was evaporated. A part (100 mg) of the residue (5.6 g; pure according to TLC) was purified by chromatography on 4 preparative TLC plates in light petroleum–ether (7 : 3) to give 70 mg (78%) of hydroxy derivative **3d**. IR spectrum: 3 615, 3 500 (hydroxyl); 3 058, 3 014 (cyclopropane); 1 727, 1 194, 1 162 (ester). ^{1}H NMR spectrum: 0.29 dd, 1 H, J = 5, J' = 8.2, and 0.52 t, 1 H, J = 4 (two cyclopropane protons); 0.69 s, 3 H (3 × H-18); 1.05 s, 3 H (3 × H-19); 1.13 d, 3 H, J = 5.8 (3 × H-21); 1.147 d and 1.153 d, 2 × H, J = 7 ($(C\mathbf{H}_3)_2CHCO$); 2.47 m, 1 H, $W_{1/2}$ = 14 (1 × H-7); 3.26 m, 1 H, $W_{1/2}$ = 6 (H-6α); 4.83 mt, 1 H, $W_{1/2}$ = 16 (H-20). For $C_{25}H_{40}O_3$ (388.6) calculated: 77.27% C, 10.38% H; found: 77.44% C, 10.33% H.

(20*R*)-20-Hydroxy-3α,5-cyclo-5α-pregnan-6-one Heptafluorobutyrate (**4a**)

Jones reagent was added to a solution of alcohol **3a** (200 mg, 0.39 mmol) in acetone (10 ml) to constant brown coloration. After standing for 10 min at room temperature, methanol (0.5 ml) was added, the mixture was set aside for 5 min, poured into water and extracted with chloroform. The chloroform extract was washed with water, saturated solution of potassium hydrogen carbonate, again water, and dried over sodium sulfate. The solvent was evaporated and the residue (200 mg) was purified by chromatography on a column of silica gel (40 g) in light petroleum–ether (4 : 1) to give 169 mg (85%) of noncrystalline keto derivative **4a**. IR spectrum: 3 070 (cyclopropane); 1 776, 1 238 (ester); 1 691 (ketone). ^{1}H NMR spectrum: 0.72 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 1.29 d, 3 H, J = 6 (3 × H-21); 2.71–2.64 m, 2 H (2 × H-7); 5.07 m, 1 H, $W_{1/2}$ = 18 (H-20). For $C_{25}H_{31}F_7O_3$ (512.5) calculated: 58.59% C, 6.09% H, 25.95% F; found: 58.66% C, 6.11% H, 26.33% F.

(20*R*)-20-Hydroxy-3α,5-cyclo-5α-pregnan-6-one Hemisuccinate (**4b**)

Alcohol **3b** (250 mg, 0.60 mmol) was oxidized in acetone (2.5 ml) with Jones reagent in the same manner as described in the preceding experiment. Analogous work-up gave 160 mg (63%) of keto derivative **4b**, m.p. 138–139 °C (ethanol). IR spectrum: 3 531, 3 500–2 400, 1 714, 1 379 (COOH); 3 024, 3 008 (cyclopropane); 1 732, 1 170 (ester); 1 690 (6-ketone). ^{1}H NMR spectrum: 0.62–0.79 m, 1 H (cyclopropane proton); 0.67 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 1.14 d, 3 H, J = 6 (3 × H-21); 2.60 m, 4 H, $W_{1/2}$ = 9 (OOCCH$_2$CH$_2$COO); 4.88 m, 1 H, $W_{1/2}$ = 19 (H-20). For $C_{25}H_{36}O_5$ (416.6) calculated: 72.08% C, 8.71% H; found: 71.99% C, 9.11% H.

(20*R*)-20-Hydroxy-3α,5-cyclo-5α-pregnan-6-one Butyrate (**4c**)

Alcohol **3c** (160 mg, 0.41 mmol) was oxidized with Jones reagent in acetone (5 ml) in the same manner as described for the preparation of compound **4a**. Analogous work-up gave 150 mg of the crude product which was purified on 4 preparative silica gel plates (elution with light petroleum–ether 7 : 3) to give 90 mg (56%) of keto derivative **4c**. IR spectrum: 3 124, 3 007 (cyclopropane); 1 731, 1 187, 1 058 (ester); 1 690 (ketone). ^{1}H NMR spectrum: 0.72 t, 1 H, J = 4.6 (cyclopropane proton); 0.68 s, 3 H (3 × H-18); 0.95 t, 3 H, J = 7.3 (ester CH$_3$); 1.00 s, 3 H (3 × H-19); 1.15 d, 3 H, J = 6.1 (3 × H-21); 2.17–2.31 m, 1 H and 2.32–2.54 m, 1 H (2 × H-7); 4.86 m, 1 H, $W_{1/2}$ = 16 (H-20). For $C_{25}H_{38}O_3$ (386.6) calculated: 77.68% C, 9.91% H; found: 77.83% C, 9.90% H.

(20*R*)-20-Hydroxy-3α,5-cyclo-5α-pregnan-6-one Isobutyrate (**4d**)

Alcohol **3d** (5 g, 12.87 mmol) was oxidized with Jones reagent in acetone (100 ml) in the same manner as described for the preparation of compound **4a**. Analogous work-up (extraction with ether) gave 5 g of the crude product which was purified by column chromatography on silica gel in light petroleum–ether (9 : 1) to give 4.2 g (84%) of keto derivative **4d**. IR spectrum: 3 024, 3 007 (cyclopropane); 1 731, 1 192, 1 160 (ester); 1 690 (ketone). ^{1}H NMR spectrum: 0.72 t, 1 H, J = 4.2 (cyclopropane proton); 0.68 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 1.14 d, 3 H, J = 6.1 (3 × H-21); 1.154 d and 1.162 d, 2 × 3 H, J = 7 ((C**H**$_3$)$_2$CHCO); 2.60–2.31 m, 2 H (2 × H-7); 4.85 m, 1 H, $W_{1/2}$ = 16 (H-20). For $C_{25}H_{38}O_3$ (386.6) calculated: 77.68% C, 9.91% H; found: 77.88% C, 9.77% H.

(20*R*)-20-Hydroxy-5α-pregn-2-en-6-one Heptafluorobutyrate (**5a**)

4-Toluenesulfonic acid monohydrate (340 mg, 2.29 mmol) was added to a solution of derivative **4a** (170 mg, 0.33 mmol) in sulfolane (3 ml). The mixture was heated at 155 °C for 1 h under nitrogen, cooled, poured into water and the product was taken up in ether. The ethereal extract was washed successively with water, saturated solution of potassium hydrogen carbonate and water, dried, and the solvent was evaporated. The residue (140 mg) was chromatographed on a column of silica gel (20 g) in light petroleum–ether (9 : 1). Yield 69 mg (39%) of oily olefin **5a**. IR spectrum: 3 030, 1 659 (C=C); 1 777, 1 238 (ester); 1 714 (ketone). ^{1}H NMR spectrum: 0.68 s, 3 H (3 × H-19); 0.72 s, 3 H (3 × H-18); 1.29 d, 3 H, J = 7 (3 × H-21); 5.07 m, 1 H, $W_{1/2}$ = 18 (H-20); 5.39–5.82 m, 2 H (H-2 and H-3). For $C_{25}H_{31}F_7O_3$ (512.5) calculated: 58.59% C, 6.09% H, 25.95% F; found: 58.31% C, 5.92% H, 24.98% F.

(20*R*)-20-Hydroxy-5α-pregn-2-en-6-one Hemisuccinate (**5b**)

Pyridinium 4-toluenesulfonate (9.4 mg) and lithium bromide (10.6 mg, 0.12 mmol) were added to a solution of derivative **4b** (100 mg, 0.24 mmol) in *N*,*N*-dimethylacetamide (1.0 ml). After heating at 160 °C for 6 h under nitrogen, the reaction mixture was cooled, poured into water and the product

was worked up in the usual manner (extraction with chloroform). After evaporation of chloroform, the residue (100 mg) was purified by chromatography on 4 preparative silica gel plates (elution with light petroleum–ether–acetic acid 60 : 40 : 1). The corresponding zones gave 86 mg (87%) of oily olefin **5b**, m.p. 137–138 °C (acetone–heptane). IR spectrum: 3 500–2 400, 1 313, 1 227 (COOH); 3 026, 1 227 (COOH); 3 026, 1 657 (C=C); 1 733, 1 174 (ester); 1 713 (ketone). ^{1}H NMR spectrum: 0.71 s, 3 H (3 × H-19); 0.63 s, 3 H (3 × H-18); 1.15 d, 3 H, J = 6.5 (3 × H-21); 2.62 m, 4 H, $W_{1/2}$ = 9 ($OOCCH_2CH_2COO$); 4.89 m, 1 H, $W_{1/2}$ = 19 (H-20); 5.61 m, 2 H, $W_{1/2}$ = 9 (H-2 and H-3). For $C_{25}H_{36}O_5$ (416.6) calculated: 72.08% C, 8.71% H; found: 71.93% C, 8.52% H.

(20*R*)-20-Hydroxy-5α-pregn-2-en-6-one Butyrate (**5c**)

Derivative **4c** (100 mg, 0.26 mmol) in dimethylacetamide (3 ml) was treated with pyridinium 4-toluenesulfonate (9.4 mg, 0.05 mmol) and lithium bromide (10.6 mg, 0.12 mmol) as described in the preceding experiment. The same work-up (extraction with ether) and purification on two preparative plates (elution with light petroleum–ether 7 : 3) afforded 45 mg (46%) of noncrystalline olefin **5c**. IR spectrum: 3 080, 1 693, 1 698 (C=C); 1 731, 1 185 (ester); 1 713 (ketone). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-19); 0.71 s, 3 H (3 × H-18); 0.95 t, 3 H, J = 7.3 (ester CH_3); 1.14 d, 3 H, J = 6.1 (3 × H-21); 4.86 m, 1 H, $W_{1/2}$ = 15 (H-20); 5.48–5.76 m, 2 H (H-2 and H-3). For $C_{25}H_{38}O_3$ (386.6) calculated: 77.68% C, 9.91% H; found: 77.51% C. 9.63% H.

(20*R*)-20-Hydroxy-5α-pregn-2-en-6-one Isobutyrate (**5d**)

Derivative **4d** (1.5 g, 3.88 mmol) in *N*,*N*-dimethylacetamide (3 ml) was treated with pyridinium 4-toluenesulfonate (140 mg, 0.73 mmol) and lithium bromide (160 mg, 1.84 mmol) as described in the preceding experiment. An analogous work-up (extraction with ether) afforded 1.2 g of residue which was purified on a column of silica gel (300 g, elution with light petroleum–ether 9 : 1). Yield 995 mg (66%) of olefin **5d**, m.p. 85–87 °C (acetone–heptane). IR spectrum: 3 080, 1 695, 1 699 (C=C); 1 731, 1 186 (ester); 1 714 (ketone). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-19); 0.71 s, 3 H (3 × H-18); 1.14 d, 3 H, J = 6.1 (3 × H-21); 1.161 d and 1.170 d, 2 × 3 H, J = 7 ($(C\mathbf{H}_3)_2CHCO$); 4.85 m, 1 H, $W_{1/2}$ = 18 (H-20); 5.30–5.75 m, 2 H (H-2 and H-3). For $C_{25}H_{38}O_3$ (386.6) calculated: 77.68% C, 9.91% H; found: 77.44% C. 9.70% H.

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

A solution of OsO_4 (24 mg, 0.09 mmol) in 2-methyl-2-propanol (0.24 ml), followed by *N*-methylmorpholine *N*-oxide (480 mg, 4.10 mmol) in water (0.5 ml), was added to a solution of olefin **5a** (480 mg, 0.94 mmol) in acetone (24 ml) and the mixture was stirred at room temperature for 5 h. Then 10% aqueous solution of sodium sulfite (12 ml) was added and stirring was continued for 1 h. The mixture was poured into water and the product was taken up in chloroform in the usual manner to give 550 mg of an oily product. Purification by chromatography on silica gel (200 g) in chloroform–ether (1 : 1) afforded 329 mg (40%) of dihydroxy derivative **6a**, subliming at 200 °C after change of modification at 142 °C. IR spectrum: 3 526, 3 415, 3 303 (OH); 1 773, 1 236, 1 216 (ester); 1 711 (ketone). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 0.76 s, 3 H (3 × H-19); 1.30 d, 3 H, J = 6 (3 × H-21); 3.74 m, 1 H, $W_{1/2}$ = 23 (H-2β); 4.02 m, 1 H, $W_{1/2}$ = 9 (H-3β); 5.07 m, 1 H, $W_{1/2}$ = 20 (H-20). Mass spectrum (*m*/*z*): 546 (M^+); 531 (M – CH_3); 528 (M – H_2O); 513 (M – CH_3 – H_2O). For $C_{25}H_{33}F_7O_5$ (546.5) calculated: 54.94% C, 6.09% H, 24.33% F; found: 55.45% C, 6.69% H, 24.18% F.

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

Olefin **5b** (130 mg, 0.31 mmol) in acetone (6.5 ml) was treated with a solution of OsO_4 (6.5 mg, 0.03 mmol) in 2-methyl-2-propanol (0.065 ml) and *N*-methylmorpholine *N*-oxide (130 mg, 1.11 mmol) in water (0.13 ml) as described in the preceding experiment. Analogous work-up afforded 150 mg of an oily product which was purified by chromatography on 4 preparative plates of silica gel in chlorofom–2-propanol–acetic acid (90 : 10 : 1) to give 48 mg (35%) of dihydroxy derivative **6b**, m.p. 248–255 °C (acetone–light petroleum). IR spectrum: 3 619, 3 569, 3 518 (OH); 3 348, 2 452, 1 713 (COOH); 1 730 (ester); 1 713 (ketone). ^{1}H NMR spectrum: 0.63 s, 3 H (3 × H-18) and 0.75 s, 3 H (3 × H-19); 1.16 d, 3 H, J = 6 (3 × H-21); 2.60 m, 4 H, $W_{1/2}$ = 3 ($OOCCH_2CH_2COO$); 3.40–3.82 m, 1 H (H-2β); 3.98 m, 1 H, $W_{1/2}$ = 7 (H-3β); 4.86 m, 1 H, $W_{1/2}$ = 18 (H-20). Mass spectrum (*m*/*z*): 450 (M); 432 (M – H_2O); 414 (M – 2 × H_2O). For $C_{25}H_{38}O_7$ (450.6) calculated: 66.64% C, 8.50% H; found: 66.41% C, 8.32% H.

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

Olefin **5c** (600 mg, 1.55 mmol) in acetone (30 ml) was treated with a solution of OsO_4 (30 mg, 0.12 mmol) in 2-methyl-2-propanol (0.3 ml) and *N*-methylmorpholine *N*-oxide (600 mg, 5.12 mmol) in water (0.6 ml) as described in the preceding experiment. Analogous work-up afforded 0.7 g of an oily product which was purified by chromatography on a column of silica gel in chlorofom–2-propanol (9 : 1). Crystallization from aqueous acetone afforded 27 mg (41%) of dihydroxy derivative **6c**, m.p. 98–101 °C. IR spectrum: 3 423 (OH); 1 730, 1 186 (ester); 1 713 (ketone). ^{1}H NMR spectrum: 0.63 s, 3 H (3 × H-18); 0.75 s, 3 H (3 × H-19); 0.97 t, 3 H, J = 7.3 (ester CH_3); 1.15 d, 3 H, J = 5.8 (3 × H-21); 2.69 dd, 1 H, J = 4, J' = 12 (H-5α); 3.76 m, 1 H, $W_{1/2}$ = 24 (H-2β); 4.04 m, 1 H, $W_{1/2}$ = 7 (H-3β); 4.84 m, 1 H, $W_{1/2}$ = 18 (H-20). Mass spectrum (FAB, *m*/*z*): 421 (M + 1); 403 (M + 1 – H_2O); 385 (M + 1 – 2 × H_2O); 333 (M + 1 – $CH_3CH_2CH_2COOH$). For $C_{25}H_{40}O_5$ (420.6) calculated: 71.39% C, 9.59% H; found: 71.04% C, 9.42% H.

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

Olefin **5d** (1.2 g, 3.10 mmol) in acetone (60 ml) was treated with a solution of OsO_4 (60 mg, 0.24 mmol) in 2-methyl-2-propanol (0.6 ml) and *N*-methylmorpholine *N*-oxide (1.2 g, 10.24 mmol) in water (1.2 ml) as described in the preceding experiment. Analogous work-up afforded 1.2 g of an oily product which was purified by chromatography on a column of silica gel in chlorofom–2-propanol (9 : 1). Crystallization from aqueous acetone afforded 250 mg (19%) of dihydroxy derivative **6d**, m.p. 115–120 °C. IR spectrum: 3 619 (OH); 1 709 (ketone and ester). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 0.73 s, 3 H (3 × H-19); 1.10 d, 3 H, J = 5.8 (3 × H-21); 1.11 d, 3 H and 1.12 d, 3 H, J = 7.0 ((CH_3)$_2$CHCO); 2.67 dd, 1 H, J = 4, J' = 12 (H-5α); 3.54–3.72 m, 1 H (H-2β); 3.90 m, 1 H, $W_{1/2}$ = 6.5 (H-3β); 4.80 m, 1 H, $W_{1/2}$ = 18 (H-20). Mass spectrum (FAB, *m*/*z*): 421 (M + 1); 403 (M + 1 – H_2O); 385 (M + 1 – 2 × H_2O); 333 (M + 1 – $(CH_3)_2CHCOOH$). For $C_{25}H_{40}O_5$ (420.6) calculated: 71.39% C, 9.59% H; found: 71.59% C, 9.30% H.

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

To a solution of ketone **6a** (210 mg, 0.38 mmol) in dichloromethane (3 ml) was added a solution of trifluoroperacetic acid, freshly prepared from trifluoroacetic anhydride (646 mg, 3.08 mmol) and hydrogen peroxide (30%, 105 mg) in dichloromethane (3 ml). After standing at room temperature for 1 h, the reaction mixture was poured into water and taken up in chloroform. The chloroform extract was washed with water, saturated solution of potassium hydrogen carbonate, water, and dried over sodium sulfate. Removal of the solvent in vacuo gave 112 mg of a residue which contained two products (TCL). The residue was chromatographed on 6 preparative plates in chloroform–2-propanol (9 : 1).

Work-up of the lipophilic zones gave 56 mg (26%) of lactone **7a**, m.p. 160–165 °C (aqueous ethanol). IR spectrum: 3 412, 1 065 (OH); 1 773, 1 236, 1 218 (ester); 1 728, 1 711, 1 186 (lactone). ^{1}H NMR spectrum: 0.71 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 1.28 d, 3 H, J = 6 (3 × H-21); 2.97–3.26 m, 1 H (H-5α); 3.75 m, 1 H, $W_{1/2}$ = 22 (H-2β); 4.07 m, 3 H, $W_{1/2}$ = 8 (H-3β and 2 × H-7a); 5.087 m, 1 H, $W_{1/2}$ = 18 (H-20). Mass spectrum (*m*/*z*): 562 (M^+); 544 (M – H_2O). For $C_{25}H_{33}F_7O_6$ (562.5) calculated: 53.38% C, 5.91% H, 23.64% F; found: 53.18% C, 6.05% H, 23.10% F.

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

Ketone **6b** (65 mg, 0.14 mmol) in dichloromethane (3 ml) was treated with a solution of trifluoroperacetic acid for 5 h as described in the preceding experiment. Analogous work-up and thin-layer chromatography on 4 preparative plates in chloroform–2-propanol–acetic acid (90 : 10 : 1) afforded 9.5 mg (14%) of pure lactone **7b**. IR spectrum ($CHCl_3$): 3 620, 3 570, 3 520 (OH); 3 400–2 400, 1 714 (COOH); 1 733 (ester); 1 728 (lactone). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-18); 0.92 s, 3 H (3 × H-19); 1.16 d, 3 H, J = 6 (3 × H-21); 3.70 m, 1 H, $W_{1/2}$ = 21 (H-2β); 4.02 m, 3 H, $W_{1/2}$ = 7 (H-3β and 2 × H-7a); 4.84 m, 1 H, $W_{1/2}$ = 18 (H-20). For $C_{25}H_{38}O_8$ (466.6) calculated: 64.36% C, 8.21% H; found: 65.77% C, 8.44% H.

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

Ketone **6c** (120 mg, 0.29 mmol) in dichloromethane (2.4 ml) was treated with a solution of trifluoroperacetic acid as described in the preceding experiment. Analogous work-up and chromatography on 6 preparative plates in chloroform–acetone (4 : 1) afforded 72 mg (55%) of lactone **7c**. IR spectrum: 3 429 (OH); 1 731 (ester and lactone). ^{1}H NMR spectrum: 0.66 s, 3 H (3 × H-18); 0.92 s, 3 H (3 × H-19); 0.95 t, 3 H, J = 7 (ester CH_3); 1.14 s, 3 H, J = 6 (3 × H-21); 3.14 dd, 1 H, J = 4, J' = 12 (H-5α); 3.75 dm, 1 H, J = 12, $W_{1/2}$ = 11 (H-2β); 4.06 m, 3 H, $W_{1/2}$ = 9 (H-3β); 4.09 m, 2 H, J = 5 (2 × H-7a); 4.87 m, 1 H, $W_{1/2}$ = 18.5 (H-20). Mass spectrum (FAB, DMSO, *m*/*z*): 459 (M + Na); 437 (M + 1); 419 (M + 1 – H_2O); 401 (M + 1 – 2 × H_2O); 349 (M + 1 – $CH_3CH_2CH_2COOH$). For $C_{25}H_{40}O_6$ (436.6) calculated: 68.78% C, 9.23% H; found: 68.88% C, 9.22% H.

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

Ketone **6d** (170 mg, 0.40 mmol) in dichloromethane (5.1 ml) was treated with a solution of trifluoroperacetic acid as described in the preceding experiment. Analogous work-up and chromatography on 7 preparative plates in chloroform–acetone (4 : 1) afforded 89 mg (50%) of lactone **7d**. IR spectrum ($CHCl_3$): 3 500 (OH); 1 720 (ester and lactone). ^{1}H NMR spectrum: 0.66 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 1.13 d, 3 H, J = 6 (3 × H-21); 1.14 d, 3 H and 1.15 d, 3 H, J = 7 ((C**H**$_3$)$_2$CHCO); 3.12 dd, 1 H, J = 4, J' = 12 (H-5α); 3.71 m, 1 H, $W_{1/2}$ = 22 (H-2β); 4.01 m, 3 H, $W_{1/2}$ = 8 (H-3β); 4.07 m, 2 H, J = 5 (2 × H-7a); 4.84 m, 1 H, $W_{1/2}$ = 18.5 (H-20). Mass spectrum (FAB, DMSO, *m*/*z*): 459 (M + Na); 437 (M + 1); 419 (M + 1 – H_2O); 401 (M + 1 – 2 × H_2O); 349 (M + 1 – $(CH_3)_2CHCOOH$). For $C_{25}H_{40}O_6$ (436.6) calculated: 68.78% C, 9.23% H; found: 68.99% C, 9.11% H.

(20*R*)-2α,3α,20-Trihydroxy-B-homo-6-oxa-5α-pregnan-7-one 20-Heptafluorobutyrate (**8**)

Work-up of the polar zones on preparative plates in the preparation of lactone **7a** afforded 37 mg (18%) of lactone **8**. IR spectrum: 3 408 (OH); 1 774, 1 236, 1 218 (ester); 1 729, 1 709 (lactone). ^{1}H NMR spectrum: 0.95 s, 6 H (3 × H-18 and 3 × H-19); 1.45 d, 3 H, J = 5 (3 × H-21); 3.73 m, 1 H, $W_{1/2}$ = 21 (H-2β); 4.03 m, 1 H, $W_{1/2}$ = 11 (H-3β); 4.54 m, 1 H, $W_{1/2}$ = 15 (H-5α); 5.01 m, 1 H, $W_{1/2}$ = 18

(H-20). Mass spectrum (*m*/*z*): 562 (M^+); 544 (M – H_2O). For $C_{25}H_{33}F_7O_6$ (562.5) calculated: 53.38% C, 5.91% H, 23.64% F; found: 53.10% C, 5.51% H, 23.17% F.

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