SYNTHESIS OF (20*S*)-2α,3α-DIHYDROXY-6-OXO-7-OXA-7a-HOMO-5α-PREGNANE-20-CARBOXYLIC ACID AS A BRASSINOSTEROID PART OF LIGAND FOR BINDING TO AFFINITY CHROMATOGRAPHY CARRIERS+

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(20*S*)-2α,3α-Dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylic acid (**12**) as a brassinosteroid ligand for affinity chromatography carriers was synthesized from bisnorcholenic acid. The brassinolide activity in the bean second internode bioassay of the synthesized (20*S*)-5α-pregnane-20-carboxylic acid derivatives is also described.

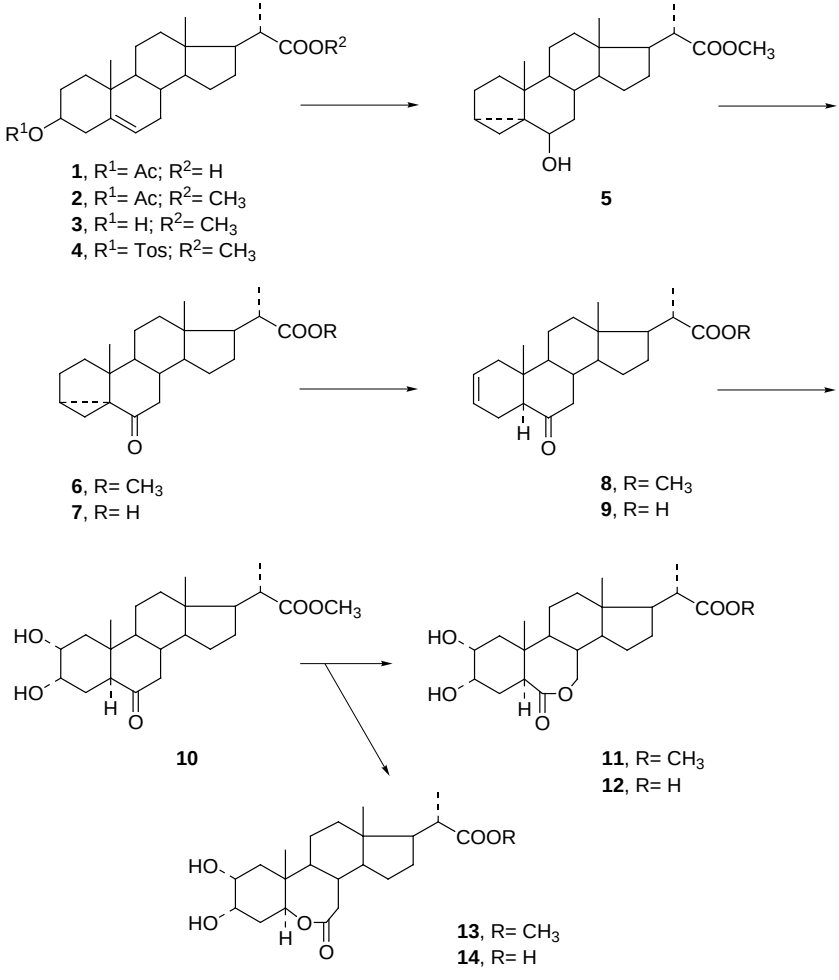
Key words: Steroids; Brassinolide; Brassinosteroids; NMR spectroscopy; Plant growth activity; Affinity carrier ligand.

In the course of our investigation[2–4] on structure–activity relationships of brassinosteroids, we turned our attention to brassinolide analogues of (20*S*)-5α-pregnane-20-carboxylic acid. These compounds with convenient spacers may find use as ligands for covalent binding to affinity chromatography carriers potentially useful for protein receptor isolation from plant extracts.

The cheapest starting material for the synthesis is commercially available (20*S*)-3β-acetoxy-5-pregnen-20-carboxylic acid[5] (**1**; *i.e.* bisnorcholenic acid acetate). Acid **1** was converted with diazomethane into its methyl ester **2**, which on saponification afforded the known 3β-alcohol **3**. To obtain tosylate **4**, we used a procedure similar to that described previously[6]. The

+ Part CDX in the series On Steroids. Part CDIX see ref.[1]

subsequent steps of the synthesis were modified in the following way: tosylate **4** was transformed into methyl (20*S*)-6β-hydroxy-3α,5-cyclo-5α-pregnane-20-carboxylate (**5**) on treatment with potassium acetate and derivative **5** was immediately oxidized with the Jones reagent to the known[6] methyl (20*S*)-6-oxo-3α,5-cyclo-5α-pregnane-20-carboxylate (**6**). To open the cyclopropane ring, we treated compound **6** with lithium bromide in *N*,*N*-dimethylacetamide in the presence of pyridinium tosylate at 160 °C. This reaction directly afforded methyl (20*S*)-6-oxo-5α-pregn-2-ene-20-carboxylate (**8**). The sequence proved to be simpler and afforded higher yields



1, R^1= Ac; R^2= H
2, R^1= Ac; R^2= CH_3
3, R^1= H; R^2= CH_3
4, R^1= Tos; R^2= CH_3

5

6, R= CH_3
7, R= H

8, R= CH_3
9, R= H

10

11, R= CH_3
12, R= H

13, R= CH_3
14, R= H

SCHEME 1

than the route described in ref.[6] (yield 68% *versus* 16%). The reaction was also applied to (20*S*)-6-oxo-3α,5-cyclo-5α-pregnane-20-carboxylic acid (**7**) obtained by alkaline hydrolysis of methyl ester **6**, simply affording (20*S*)-6-oxo-5α-pregn-2-ene-20-carboxylic acid (**9**). Osmium tetroxide catalyzed hydroxylation of olefin **8** with 4-methylmorpholine *N*-oxide resulted in the formation of 2α,3α-diol **10**. This diol was directly treated with trifluoroperoxyacetic acid in dichloromethane (as we described previously[7]) without any protection of the diol groups to yield the products of Baeyer–Villiger oxidation as a mixture of methyl (20*S*)-2α,3α-dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylate (**11**) and methyl (20*S*)-2α,3α-dihydroxy-6-oxa-7-oxo-7a-homo-5α-pregnane-20-carboxylate (**13**) (Scheme 1). The latter compound could not be isolated at this step but it was detected by the corresponding signals in the NMR spectra of mother liquors from the crystallization of lactone **11**. Alkaline hydrolysis of this mixture afforded (20*S*)-2α,3α-dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylic acid (**12**) and (20*S*)-2α,3α-dihydroxy-6-oxa-7-oxo-7a-homo-5α-pregnane- 20-carboxylic acid (**14**). (20*S*)-2α,3α-Dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylic acid (**12**) was obtained in 25% overall yield based on the starting (20*S*)-3β-acetoxypregn-5-ene-20-carboxylic acid (**1**). Derivative **12** was used as a brassinosteroid ligand[8] with a convenient spacer and bound covalently to an affinity chromatography carrier for protein isolation from plant extracts. The results on protein receptor isolation will be published in due course.

TABLE I
Lengthening of the second internode[a] (mm) in the bean second internode assay of lactone **12** and 24-epiBR[b]

Compound	Amount applied, mole					
	$1 \cdot 10^{-7}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-11}$	$1 \cdot 10^{-12}$
24-EpiBR	NT[c]	20.4	30.8	32.3	18.6	12.1
Lactone **12**	6.6	7.6	8.9	12.0	9.0	8.0

[a] The lengthening of the second internode means its lengthening in mm as compared with that of the reference plant for the applied amount given. [b] 24-epiBR means 24-epibrassinolide, *i.e.* (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7a-homo-5α-cholestan-6-one. [c] Not tested.

The brassinolide activities of the compound synthesized were measured by a modified bean second internode bioassay as described in Experimental. Of the tested compounds **10**–**14**, the highest activity was found for lactone **12**; however, even this compound exhibited a much lower activity than the natural 24-epibrassinolide [(22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7a-homo-5α-cholestan-6-one] (Table I).

EXPERIMENTAL

The melting points were determined on a micro melting point Electrothermal (U.S.A.). Optical rotations were measured at 25 °C in chloroform (unless otherwise stated) and $[\alpha]_D^{25}$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Bruker IFS 88 spectrometer in tetrachloromethane (unless otherwise stated), wavenumbers are given in cm^{-1}. ^{1}H NMR spectra were taken in deuteriochloroform on a Varian XL-200 (FT mode, 200.04 MHz) instrument in deuteriochloroform solutions with tetramethylsilane as an internal reference unless otherwise stated. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and multiplet half-width ($W_{1/2}$) in Hz. All values were obtained by first-order analysis. Mass spectra were obtained with a ZAB-EG spectrometer at 70 eV. The identity of the prepared samples was checked by melting points, thin-layer chromatography (TLC) performed on silica gel G (ICN Biochemicals, the detection was carried out by spraying with sulfuric acid and heating), IR and ^{1}H NMR spectra. Preparative TLC was carried out on 200 × 200 mm plates coated with 0.7 mm layer of silica gel Woelm DC, the detection by spraying the plates with a 0.2% methanolic morine solution, by UV detection or by spraying with sulfuric acid and heating the side strips of the plates. For column chromatography, neutral silica gel 60–120 μm was used (Service Laboratories of the Institute).

"Usual" work-up means extraction with a given organic solvent, washing the organic phase with 5% hydrochloric acid, water, 5% aqueous potassium hydrogencarbonate, water, drying over anhydrous sodium sulfate, filtering and evaporation of the solvent to dryness under reduced pressure.

Light petroleum refers to a fraction boiling at 40–62 °C.

Bean Second Internode Bioassay

Seeds of bean (*Phaseolus vulgaris* L., var. Pinto) were germinated for two days and selected seeds were planted into pots containing perlite and modified Hoagland's solution (half concentration, pH 5.7). The pots were placed in light-controlled cultivation room (25–27 °C, light 48 W/m^2, light/dark period 16 h/8 h). Groups of eight 7-day-old bean seedlings with 1–2 mm long second internodes were treated with different ammounts of the tested compounds in 2 μl of lanolin. Control plants were treated only with lanolin. The measurements were done after 5 days. The difference in the length of the second internode of treated and control plants was used as a measure of the activity.

Methyl (20*S*)-3β-Acetoxypregn-5-ene-20-carboxylate (**2**)

(20*S*)-3β-Acetoxypregn-5-ene-20-carboxylic acid[5] (**1**; 3.9 g, 10 mmol) was dissolved in ether (100 ml). A solution of diazomethane (1.26 g, 30 mmol) in ether (48 ml) was added. The re-

action mixture was poured into water after 10 min and then worked up as usual. Pure methyl ester **2** (4.25 g; 100%) was obtained. Analytical sample was crystallized from acetone: m.p. 144–146 °C, $[\alpha]_D^{25}$ –42 (*c* 1.14) in accordance with literature[9]. IR: 3 060, 1 668 (C=C), 1 735 (C=O acetate and ester), 1 434 (MeO), 1 215, 1 033 (C–O acetate), 1 196, 1 160 (C–O methyl ester). ^{1}H NMR: 0.69 s, 3 H (3 × H-18); 1.02 s, 3 H (3 × H-19); 1.19 d, 2 H, *J* = 6.7 (3 × H-21); 2.03 s, 3 H (CH_3COO); 3.645 s, 3 H (CH_3OOC); 4.60 m, 1 H, $W_{1/2}$ = 24 (H-3α); 5.37 br d, $W_{1/2}$ = 8, *J* = 4.6 (H-6).

Methyl (20*S*)-3β-Hydroxypregn-5-ene-20-carboxylate (**3**)

A solution of methyl (20*S*)-3β-acetoxypregn-5-ene-20-carboxylate (**2**; 2.01 g, 5 mmol) and potassium hydrogencarbonate (1.8 g, 18 mmol) in methanol (450 ml) and water (23 ml) was heated at reflux for 90 min. After evaporation of the solvent, the mixture was diluted with water and ether, the ethereal layer was separated, washed with water and dried (Na_2SO_4). Evaporation *in vacuo* afforded pure product **3** in 99% yield (1.8 g). Analytical sample was crystallized from methanol: m.p. 141–142 °C, $[\alpha]_D^{25}$ –45 (*c* 1.25), giving identical physical constants as described in the literature[10]. IR: 3 622, 3 496 (OH), 3 028, 1 668 (C=CH), 1 737 (C=O), 1 435 (OMe), 1 194 (C–OH), 1 162, 1 054 (C–O ester). ^{1}H NMR: 0.70 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19); 1.19 d, 3 H, *J* = 7.0 (3 × H-21); 2.44 m, 1 H, $W_{1/2}$ = 8 (H-20); 3.52 m, 1 H, $W_{1/2}$ = 24 (H-3α); 3.65 s, 3 H (CH_3OOC); 5.36 br d, *J* = 5.5, $W_{1/2}$ = 8.5 (H-6).

Methyl (20*S*)-3β-(4-Toluensulfonyloxy)pregn-5-ene-20-carboxylate (**4**)

Methyl (20*S*)-3β-hydroxypregn-5-ene-20-carboxylate (**3**; 2.3 g, 6.4 mmol) was dissolved in pyridine (23 ml) and the solution was treated with tosyl chloride (2.3 g, 12 mol). After six days, the mixture was worked up as usual into chloroform. Evaporation *in vacuo* afforded 2.72 g (83%) of product **4**. Analytical sample was dissolved in chloroform and ether was added. Crystals obtained on standing melted at 140–142 °C (dec.), which is in agreement with the literature value[6]. IR: 3 065, 3 033 (Tos, C=C), 1 736 (C=O), 1 600, 1 495, 1 395 (C_6H_4-ring), 1 379, 1 178, 568, 556 (SO_2), 1 161 (C–O ester). ^{1}H NMR: 0.67 s, 3 H (3 × H-18); 0.97 s, 3 H (3 × H-19); 1.18 d, 3 H, *J* = 6.7 (3 × H-21); 2.49 s, 3 H (CH_3 tosylate); 3.64 s, 3 H (CH_3OOC); 4.32 m, 1 H, $W_{1/2}$ = 24 (H-3α); 5.32 br d, 1 H, *J* = 6, $W_{1/2}$ = 8.5 (H-6); 7.34 d, 2 H and 7.80 d, 2 H, *J* = 8.0 (C_6H_4 of the tosyl group).

Methyl (20*S*)-6-Oxo-3α,5-cyclo-5α-pregnane-20-carboxylate (**6**)

A mixture of tosylate **4** (935 mg, 1.8 mmol) and potassium acetate (2 g, 20 mmol) in acetone (30 ml) and water (9 ml) were heated at reflux for 6.5 h. After cooling, the reaction mixture was poured into water and worked up as usual by extraction with ether. The crude methyl (20*S*)-6β-hydroxy-3α,5-cyclo-5α-pregnane-20-carboxylate (**5**) [IR: 3 625 (OH), 3 070 (cyclopropane), 1 737, 1 160 ($COOCH_3$). ^{1}H NMR: 0.19–0.39 m, 1 H and 0.39–0.60 m, 1 H (cyclopropane protons); 0.73 s, 3 H (3 × H-18); 1.05 s, 3 H (3 × H-19); 1.22 d, 3 H, *J* = 7.0 (3 × H-21); 2.46 m, 1 H, $W_{1/2}$ = 6.2 (20-H); 3.26 m, 1 H, $W_{1/2}$ = 8 (H-6α); 3.67 s, 3 H (CH_3OOC)] was dissolved in acetone (20 ml) and treated with the Jones reagent at 5 °C until permanent orange colour persisted. The excess reagent was destroyed by the addition of propan-2-ol. After filtration, the solution was diluted with ether and worked up as usual. Evaporation of the solvents *in vacuo* afforded 860 mg of a solid residue. The residue was chromatographed on a column of silica gel (100 g); elution with light petroleum–ether (7 : 3) gave 589 mg

(91%) of pure ketone **6**. M.p. 112–113 °C (methanol–water), $[\alpha]_D^{25}$ +34 (*c* 1.19) in accordance with the literature[6]. IR: 3 070 (cyclopropane), 1 738 (C=O ester), 1 690 (6-C=O), 1 434, 1 379, 1 374 ($COOCH_3$), 1 162 (C–O). ^{1}H NMR: 0.73 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19); 1.205 d, 3 H, *J* = 7.0 (3 × H-21); 2.44 m, 1 H, $W_{1/2}$ = 6.0 (20-H); 3.65 s, 3 H (CH_3OOC).

(20*S*)-6-Oxo-3α,5-cyclo-5α-pregnane-20-carboxylic Acid (**7**)

A solution of methyl ester **6** (50 mg, 0.14 mmol) and potassium hydroxide (100 mg, 1.8 mmol) in methanol (10 ml) was heated at reflux under nitrogen for 4 h. The solution was then neutralized with 5% aqueous HCl, evaporated to dryness *in vacuo*, water was added and the product extracted with chloroform. The chloroform layer was washed with water, dried with sodium sulfate and the solvent evaporated *in vacuo*. The oily residue (50 mg) was purified by preparative TLC on silica gel (2 plates) in light petroleum–ether (1 : 1). Extraction of the zones containing the product afforded 42 mg (87%) of acid **7**, m.p. 177–179 °C (methanol–water). IR (KBr): 3 070 (cyclopropane), 2 639, 2 573 (dimer COOH), 1 739 (dimer COOH), 1 714 (monomer COOH), 1 678 (6-C=O). ^{1}H NMR: 0.75 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19); 1.26 d, 3 H, *J* = 6.7 (3 × H-21); 2.44 m, 1 H, $W_{1/2}$ = 6.0 (20-H). For $C_{22}H_{32}O_3$ (344.5) calculated: 76.70% C, 9.36% H; found: 76.34% C, 9.42% H.

Methyl (20*S*)-6-Oxo-5α-pregn-2-ene-20-carboxylate (**8**)

Pyridinium tosylate (50 mg, 0.27 mmol) and lithium bromide (50 mg, 0.57 mmol) were added to a solution of cyclopropane derivative **6** (500 mg, 1.4 mmol) in *N*,*N*-dimethylacetamide (15 ml). The reaction mixture was heated at 160 °C under nitrogen for 4 h. Further portions of pyridinium tosylate (100 mg, 0.55 mmol) and lithium bromide (100 mg, 1.13 mmol) were added to the reaction mixture and heating continued at 160 °C for another 6 h. After cooling, the mixture was poured into water, the product extracted with ether and the ethereal solution worked up as usual. The crude product (500 mg) was chromatographed on a column of silica gel (light petroleum–ether, 4 : 1) to afford 462 mg (92%) of olefin **8**, m.p. 149–150 °C (methanol) and $[\alpha]_D^{25}$ +26 (*c* 1.21) are in accordance with the literature[6]. IR: 1 738 (C=O ester), 1 712 (6-C=O), 1 687 (C=C), 1 435 (COOMe), 1 162 (C–O). ^{1}H NMR: 0.69 s, 3 H (3 × H-18); 0.71 s, 3 H (3 × H-19); 1.20 d, 3 H, *J* = 7.0 (3 × H-21); 3.65 s, 3 H (CH_3OOC); 5.63 dm, 2 H (H-2 and H-3). For $C_{23}H_{34}O_3$ (358.5) calculated: 77.05% C, 9.56% H; found: 77.19% C, 9.31% H.

(20*S*)-6-Oxo-5α-pregn-2-ene-20-carboxylic Acid (**9**)

Methyl ester **8** (40 mg, 0.11 mmol) in methanol (4 ml) was hydrolyzed in the same way as described for the preparation of compound **7**. After a similar work-up, the residue (40 mg) was purified by preparative TLC on silica gel (2 plates) in light petroleum–ether (1 : 1). The zones containing the product afforded 29 mg (77%) of acid **9**, m.p. 168–169 °C (ethanol), $[\alpha]_D^{25}$ +23 (*c* 1.11). IR (KBr): 1 742 (COOH), 1 713 (6-C=O). ^{1}H NMR: 0.70 s, 3 H and 0.71 s, 3 H (3 × H-18 and 3 × H-19); 1.24 d, 3 H, *J* = 7.5 (3 × H-21); 5.55 d, 1 H and 5.68 m, 1 H (AB system, H-2 and H-3). For $C_{22}H_{32}O_3$ (344.5) calculated: 76.70% C, 9.36% H; found: 76.41% C, 9.31% H.

Methyl (20*S*)-2α,3α-Dihydroxy-6-oxo-5α-pregnane-20-carboxylate (**10**)

A solution of olefin **8** (249 mg, 0.89 mmol) in acetone (12.5 ml) and tetrahydrofuran (12.5 ml) was mixed with a solution of osmium tetroxide (25 mg) in *tert*-butyl alcohol (0.25 ml), *N*-methylmorfoline *N*-oxide (0.4 g) and water (0.6 ml). After standing at room temperature under argon for 36 h, 10% solution of sodium sulfite (3.0 ml) was added and the mixture was stirred at room temperature for 30 min. The product was extracted with chloroform and processed in the usual manner to give 280 mg of crude material. Chromatography on a column of silica gel (ether–ethyl acetate–chloroform, 1 : 1 : 2) afforded 179 mg (51%) of diol **10**, m.p. 197–198 °C (methanol–water), $[\alpha]_D^{25}$ –3 (*c* 0.91) in accordance with the literature[6]. IR: 3 610, 3 585, 1 050 (OH), 1 730 (C=O ester), 1 714 (6-C=O), 1 175 (C–O). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.71 s, 3 H (3 × H-19); 1.19 d, 3 H, *J* = 7.0 (3 × H-21); 3.65 s, 3 H ($COOCH_3$); 3.77 m, 1 H, $W_{1/2}$ = 26 (H-2β); 4.07 m, 1 H, $W_{1/2}$ = 8 (H-3β). MS, *m/z*: 392 (M^+), 377 (M – CH_3), 374 (M – H_2O). For $C_{23}H_{36}O_5$ (392.5) calculated: 70.38% C, 9.24% H; found: 70.31% C, 9.11% H.

Methyl (20*S*)-2α,3α-Dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylate (**11**)

A solution of trifluoroperoxyacetic acid in dichloromethane (38 ml), prepared from 5 g (13 mmol) of trifluoroacetic anhydride and 0.5 ml (8 mmol) of 50% hydrogen peroxide, was added to a solution of ketone **10** (785 mg, 2.0 mmol) in dichloromethane (16 ml). After standing at room temperature for 20 h, the reaction mixture was poured into a 10% potassium hydrogencarbonate solution. The product was extracted with chloroform, the organic extract was washed with water, dried over sodium sulfate and the solvent was evaporated *in vacuo*. The residue (845 mg) was chromatographed on a column of silica gel (150 g) in a chloroform–ether–light petroleum mixture (1 : 1 : 1) to give 701 mg (86%) of a product which contained mainly compound **11**, m.p. 187–188 °C (ethanol–water), $[\alpha]_D^{25}$ +35 (*c* 1.16) in accordance with the literature[6]. IR (chloroform): 3 620 (OH), 1 730 (C=O ester), 1 723 (C=O lactone). ^{1}H NMR: 0.71 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.19 d, 3 H, *J* = 6.7 (3 × H-21); 2.55 m, 1 H (20-H); 3.65 s, 3 H ($COOCH_3$); 3.74 m, 1 H, $W_{1/2}$ = 25 (H-2β); 4.15 m, 1 H, $W_{1/2}$ = 8 (H-3β); 4.08–4.12 m, 2 H (H-7a). MS, *m/z*: 408 (M^+), 393 (M – CH_3), 390 (M – H_2O). For $C_{23}H_{36}O_6$ (408.5) calculated: 67.62% C, 8.88% H; found: 67.61% C, 8.91% H.

Methyl (20*S*)-2α,3α-Dihydroxy-6-oxa-7-oxo-7a-homo-5α-pregnane-20-carboxylate (**13**)

This 6-oxa-7-oxo-isomer **13** of compound **11** was inseparable from this substance and was identified by ^{1}H NMR: 0.73 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 3.44 dd, 1 H (H-5) as a minor component in the mother liquor after the crystallization of lactone **11**.

(20*S*)-2α,3α-Dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylic Acid (**12**)

Methyl ester **11** (820 mg, 2.01 mmol) in methanol (80 ml) with potassium hydroxide (1.2 g, 21 mmol) was heated at reflux under nitrogen for 10 h. The solution was neutralized with 5% aqueous HCl, 37% HCl (0.5 ml) in methanol (4.5 ml) was subsequently added and the reaction mixture was left to stand at room temperature for 30 min. The mixture was evaporated to dryness, water (100 ml) was added, and the product was extracted with chloroform (3 × 60 ml). The chloroform solution was dried with sodium sulfate and evaporated under reduced pressure. The residue (690 mg) was crystallized from ethanol–water to afford 317

mg of the product. The mother liquor was evaporated under reduced pressure to dryness and the residue was again crystallized from ethanol and water to afford 155 mg of product **12**. One more crystallization of the mother liquor afforded a further portion of the product, giving a total of 557 mg (70.3%) of compound **12**, m.p. 321–322 °C (ethanol–water) in accordance with the literature[6], $[\alpha]_D^{25}$ +45 (*c* 1.23, methanol). IR (KBr): 3 367, 3 142 (OH), 2 622 (dimer COOH), 1 746 (C=O lactone), 1 705 (C=O carboxyl), 1 281 (C=O of dimer COOH), 1 062, 1 039, 966 (C–OH). ^{1}H NMR (CD_3OD): 0.95 s, 3 H (3 × H-18); 1.09 s, 3 H (3 × H-19); 1.38 d, 3 H, *J* = 6.9 (3 × H-21); 2.53 m, 1 H, $W_{1/2}$ = 6 (20-H); 3.79 dm, 1 H (H-2β); 4.13 m, 1 H (H-3β); 4.20–4.44 m, 2 H (2 × H-7a); (DMSO-d_6): 0.68 s, 3 H (3 × H-18); 0.77 s, 3 H (3 × H-19); 1.07 d, 3 H, *J* = 7.0 (3 × H-21). For $C_{22}H_{34}O_6$ (394.5) calculated: 66.98% C, 8.69% H; found: 66.91% C, 8.51% H.

(20*S*)-2α,3α-Dihydroxy-6-oxo-7-oxa-7a-homo-5α-pregnane-20-carboxylic acid (**14**)

Careful separation of a part (40 mg) of the evaporated mother liquor from the crystallization of isomer **12** on preparative TLC plates on silica gel (8 plates) in chloroform–methanol (4 : 1) afforded 1.6 mg of the more polar isomer, lactone **14**. ^{1}H NMR (CD_3OD): 0.94 s, 3 H (3 × H-18); 1.13 s, 3 H (3 × H-19); 3.44 d, 1 H (H-5). FAB-MS, *m/z*: 395 $(M + 1)^+$.

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