Z-ISOMERS OF STEROID 17β-SIDE CHAIN METHYL ACRYLATES*

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THP-Protected androstan-3-ol 17β-carboxaldehydes *I – III* with 3β,5-ene, 3β,5α, and 3α,5α respective arrangements, were transformed into corresponding methyl *Z*- and *E*-acrylates *IVa – IXa*. Peterson olefination by methyl trimethylsilylacetate gave mainly *Z*-isomers, whereas under conditions of Wittig–Horner reaction with trimethyl phosphonoacetate *E*-isomers were obtained in more stereoselective manner. THP-Ethers of methyl *Z*-acrylates *IVa*, *VIa*, and *VIIIa* were deprotected and from resulting 3-hydroxy derivatives *IVb*, *VIb*, and *VIIIb* the hemisuccinates *IVd*, *VId*, and *VIIId* and β-D-glucopyranosides *IVf*, *VIf*, and *VIIIf* were prepared.

Using the Wittig–Horner reagent we recently prepared several acrylate side chain steroid derivatives starting from the corresponding 17β-formyl compounds[1]. Main products of the reaction have *E*-configuration in α,β-unsaturated ester moiety. This paper is dealing with the corresponding *Z*-isomers in methyl ester series and with the reinvestigation of before-mentioned reaction regarding the minor components. For synthesis of *Z*-acrylates we chose the Peterson olefination, which seems to be less stereoselective (cf. both reactions on steroid 20-ketones[2]), and applied it to 17β-formyl androstanes. Instead of ethyl trimethylsilylacetate we used its methyl ester in order to obtain methyl acrylates comparable with derivatives from previous work[1]. For preparation of this reagent we utilized the same method[3] as in the case of ethyl ester (see Experimental).

The Peterson olefination was tested on 3β-(2-tetrahydropyranyloxy)androst-5-ene-17β-carbaldehyde (*I*), which was reacted with lithium enolate of methyl trimethylsilylacetate in tetrahydrofuran. In resulting mixture after working up no starting compound could be detected. The products were separated by column chromatography and *Z*- and *E*-isomers *IVa* and *Va* were isolated in 30% and 11% yields, respectively. The later product was identical with authentic[1] methyl (20*E*)-3β-(2-tetrahydropyranyl-

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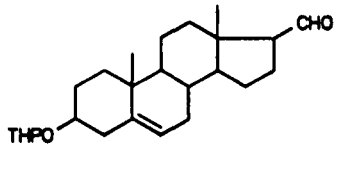
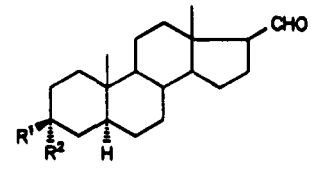
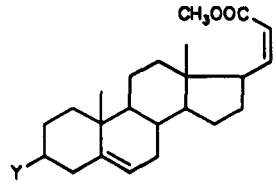
oxy)-5,20-pregnadiene-21-carboxylate (*Va*). The structure of the *Z*-isomer *IVa* was supported by its ^{1}H NMR spectrum (see Table I). In the IR spectra of esters *IVa* and *Va* the differences are discernible; bands corresponding to unsaturated ester at 1 726 and 1 654 cm^{-1} for *E*-isomer[1] *Va* are shifted to 1 723 and 1 640 cm^{-1}, respectively, in *Z*-isomer *IVa*.



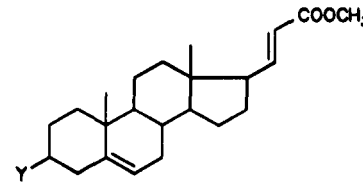
I



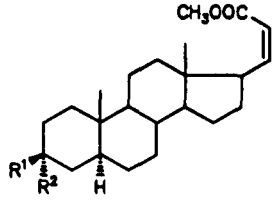
II, R^1 = OTHP; R^2 = H
III, R^1 = H; R^2 = OTHP



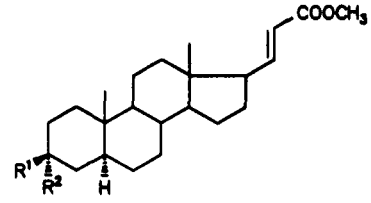
IVa – IVf



Va, *Vb*



VIa – VIf, *VIIIa – VIIIf*



VIIa, *VIIb*, *IXa*, *IXb*

In formulae
VI and *VII* : R^1 = Y; R^2 = H; *VIII* and *IX* : R^1 = H; R^2 = Y;
VI – IX : a, Y = OTHP; b, Y = OH;
c, Y = $OOCCH_2CH_2COOCH_2CH_2Si(CH_3)_3$; d, Y = $OOCCH_2CH_2COOH$;
e, Y = 2,3,4,6–tetra–O–acetyl–β–D–glucopyranosyloxy;
f, R = β–D–glucopyranosyloxy

The same procedure was used for preparation of *Z*- and *E*-isomers of acrylates derived from saturated androstane skeleton, i. e., from 3β-(2-tetrahydropyranyloxy)-5α-androstane-17β-carbaldehyde (*II*) acrylates *VIa* and *VIIa* in 32% and 10% yields and

from 3α-(2-tetrahydropyranyloxy)-5α-androstane-17β-carbaldehyde (*III*) acrylates *VIIIa* and *IXa* in 33% and 11% yields, respectively, were prepared.

With samples of both acrylate isomers in hands we reinvestigated reaction mixtures after original[1] Wittig–Horner reaction of aldehydes *I* – *III* with trimethyl phosphonoacetate. We found after column chromatography *Z*-isomers in amount of 4 – 5% beside 70 – 76% of major *E*-isomers. All isolated compounds were checked for configurational purity by reverse phase HPLC (see Table II).

For consistency with the previous work[1] and for the purpose of biological testing we prepared hemisuccinate and D-glucoside conjugates of *Z*-acrylates in series *IV*, *VI*, and *VIII*. Starting from 2-tetrahydropyranyl derivatives *IVa*, *VIa*, and *VIIIa* by deprotection with *p*-toluenesulfonic acid in benzene–methanol mixture we obtained corresponding 3-hydroxy derivatives *IVb*, *VIb*, and *VIIIb*. Their transformation to hemisuccinates *IVd*, *VId*, and *VIIId* was achieved via (2-trimethylsilyl)ethyl protected intermediates *IVc*, *VIc*, and *VIIIc* by a method developed in this Laboratory[4]. D-Glucosides *IVf*, *VIf*, and *VIIIf* were prepared from hydroxy derivatives *IVb*, *VIb*, and *VIIIb* by reaction with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide under catalysis of silver silicate[5] in 1,2-dichloroethane and by subsequent deacetylation of resulting peracetyl derivatives *IVe*, *VIe*, and *VIIIe*.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured on a Perkin–Elmer 141 MC polarimeter at 25 °C. IR spectra were taken on a Perkin–Elmer PE 580 spectrometer (wavenumbers in cm^{-1}). ^1H NMR spectra were obtained with a Tesla BS-497 (FT mode, 100 MHz) instrument at 23 °C in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. All parameters were obtained by

TABLE I
Characteristic parameters of ^1H NMR spectra of steroid acrylates *IVa* – *IXa*. Measured in $CDCl_3$; for other conditions see Experimental

Compound	Configuration	H-20	H-21	*J*(17,20)	*J*(17,21)	*J*(20,21)
IVa	20*Z*	6.17 dd	5.78 d	10.3	≈ 0	12.0
Va[a]	20*E*	6.97 dd	5.78 dd	7.8	1.5	16.0
VIa	20*Z*	6.16 dd	5.78 d	10.5	≈ 0	11.6
VIIa[a]	20*E*	6.95 dd	5.77 dd	7.6	1.0	15.6
VIIIa	20*Z*	6.16 dd	5.77 d	10.5	≈ 0	12.0
IXa[a]	20*E*	6.96 dd	5.77 dd	7.5	1.0	15.7

[a] Values taken from ref.[1].

the first-order analysis. Column chromatography was performed on silica gel (60 – 120 μm) or on neutral alumina (Reanal, activity II), thin-layer chromatography on silica gel G according to Stahl (ICN Biochemicals). HPLC analysis was carried out on Spectra–Physics instrument with 250 × 4 mm i.d. column packed with Separone Si C_{18} (Tessek, Prague), detection at 230 nm. Samples were applied as 1 mg/ml solutions (10 μl) in methanol–dichloromethane (1 : 1). Solutions in organic solvents were dried over anhydrous sodium sulfate and the solvents were evaporated in vacuo (about 2 kPa). Analytical samples were dried over phosphorus pentoxide at 40 °C/26 Pa for 12 h. The identity of samples prepared by different routes was checked by comparison of their IR and ^{1}H NMR spectra, thin-layer chromatography and mixture melting point determination.

Methyl Trimethylsilylacetate

The title compound was prepared from methyl bromoacetate (52.4 ml, 0.47 mol), trimethylsilyl chloride (64.0 ml, 0.50 mol), zinc powder (48.7 g, 0.74 mol), and copper(I) chloride (7.5 g, 76 mmol) as described for the preparation of ethyl ester[3]. Yield 36 g (49%) of product, b.p. 65 – 67 °C/6.40 kPa, literature[6] gives b.p. 65 – 68 °C/6.66 kPa. ^{1}H NMR spectrum (referenced to $CHCl_3$): 3.61 s, 3 H ($COOCH_3$); 1.89 s, 2 H ($SiCH_2COO$); 0.01 s, 9 H ($Si(CH_3)_3$).

TABLE II
HPLC of isomeric methyl acrylates *IVa – IXa* and *IVb – IXb* on Separone SGX C_{18}, retention time (t_r); for other conditions see Experimental

Compound	Configuration	Solvent A[a] t_r, min	Solvent B[b] t_r, min
		THP-derivatives	
IVa	20*Z*	6.44	16.89
Va	20*E*	6.57	17.46
VIa	20*Z*	7.01	20.73
VIIa	20*E*	7.11	21.66
VIIIa	20*Z*	7.14	23.10
*IX*a	20*E*	7.18	23.71
		Hydroxy derivatives	
IVb	20*Z*	3.73	5.33
Vb	20*E*	3.78	5.56
VIb	20*Z*	3.91	5.77
VIIb	20*E*	3.92	6.15
VIIIb	20*Z*	3.81	6.22
IXb	20*E*	3.99	7.16

[a] Methanol, flow rate 1 ml min^{-1}, pressure 6.45 MPa. [b] Ethanol–water (8 : 2), flow rate 1 ml min^{-1}, pressure 14.82 MPa.

General Procedure for Peterson Olefination of Aldehydes *I – III*

A solution of 1.6M butyllithium in hexanes (11.3 ml, 18.1 mmol) was added under argon at −78 °C to a stirred solution of diisopropylamine (2.52 ml, 18 mmol) in tetrahydrofuran (30 ml). The mixture was stirred for 30 min at −78 °C and methyl trimethylsilylacetate (2.96 ml, 18 mmol) was added. After stirring for 10 min at −78 °C a solution of aldehyde (6 mmol) in tetrahydrofuran (15 ml) was added and the stirring was continued for 1.5 h at −78 °C, 1 h at −20 °C and 1 h at 0 °C. The mixture was decomposed with saturated aqueous ammonium sulfate, the product was taken up in ether and washed with an ammonium sulfate solution (3 times). The residue was chromatographed on an alumina column (250 g) in light petroleum–ether (92 : 8).

Methyl (20*Z*)-3β-(2-Tetrahydropyranyloxy)pregna-5,20-diene-21-carboxylate (*IVa*) and Methyl (20*E*)-3β-(2-Tetrahydropyranyloxy)pregna-5,20-diene-21-carboxylate (*Va*)

The aldehyde[1] *I* (2.32 g, 6 mmol) afforded 803 mg (30%) of methyl ester *IVa* and 291 mg (11%) of methyl ester *Va* identical with authentic sample[1]. Compound *IVa*: m.p. 128 – 131 °C (hexane–ether), $[\alpha]_D$ −148° (*c* 0.3, chloroform). IR spectrum (tetrachloromethane): 1 723 (C=O); 1 640 (C=C); 1 172, 1 034 (C–O). ^{1}H NMR spectrum: 6.17 dd, 1 H (H-20, *J*(17,20) = 10.3; *J*(20,21) = 12.0); 5.78 d, 1 H (H-21, *J*(20,21) = 12.0); 5.35 bd, 1 H (H-6, *J* = 4.5); 4.71 bs, 1 H (H-2 of tetrahydropyranyloxy group); 3.70 s, 3 ($COOCH_3$); 1.01 s, 3 H (3 × H-19); 0.69 s, 3 H (3 × H-18). For $C_{28}H_{42}O_4$ (442.6) calculated: 75.98% C, 9.56% H; found: 76.18% C, 9.74%H.

Methyl (20*Z*)-3β-(2-Tetrahydropyranyloxy)-5α-pregn-20-en-21-carboxylate (*VIa*) and Methyl (20*E*)-3β-(2-Tetrahydropyranyloxy)-5α-pregn-20-en-21-carboxylate (*VIIa*)

The aldehyde[1] *II* (2.33 g, 6 mmol) gave 855 mg (32%) of methyl ester *VIa* and 255 mg (10%) of methyl ester *VIIa* identical with authentic sample[1]. Compound *VIa*: m.p. 113 – 120 °C (hexane–ether), $[\alpha]_D$ −136° (*c* 0.2, chloroform). IR spectrum (tetrachloromethane): 1 724 (C=O); 1 642 (C=C). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 11.6); 5.78 d, 1 H (H-21, *J*(20,21) = 11.6); 4.71 bs, 1 H (H-2 of tetrahydropyranyloxy group); 3.69 s, 3 H ($COOCH_3$); 0.81 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{28}H_{44}O_4$ (444.7) calculated: 75.63% C, 9.97% H; found: 76.45% C, 9.81% H.

Methyl (20*Z*)-3α-(2-Tetrahydropyranyloxy)-5α-pregn-20-en-21-carboxylate (*VIIIa*) and Methyl (20*E*)-3α-(2-Tetrahydropyranyloxy)-5α-pregn-20-en-21-carboxylate (*IXa*)

The aldehyde[1] *III* (2.33 g, 6 mmol) afforded 878 mg (33%) of methyl ester *VIIIa* and 282 mg (11%) of methyl ester *IXa* identical with authentic sample[1]. Compound *VIIIa*: oil, $[\alpha]_D$ −66° (*c* 0.2, chloroform). IR spectrum (tetrachloromethane): 1 723 (C=O); 1 642 (C=C); 1 176, 1 025, 1 008 (C–O). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 12.0); 5.77 d, 1 H (H-21, *J*(20,21) = 12.0); 4.62 bs, 1 H (H-2 of tetrahydropyranyloxy group); 3.88 m, 1 H (H-3); 3.69 s, 3 H ($COOCH_3$); 0.78 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{28}H_{44}O_4$ (444.7) calculated: 75.63% C, 9.97% H; found: 75.34% C, 10.26% H.

General Procedure for Wittig–Horner Olefination of Aldehydes *I – III*

Aldehyde (10 mmol) was allowed to react with reagent prepared from trimethyl phosphonoacetate (5.71 ml, 50 mmol) and sodium hydride (1.20 g, 50 mmol) in 1,2-dimethoxyethane (180 ml) according to ref.[1]. Products were separated on an alumina column (400 g) in light petroleum–ether (92 : 8).

Methylesters IVa *and* Va. The aldehyde[1] *I* (3.87 g, 10 mmol) afforded 197 mg (4%) of methyl ester *IVa* and 3.36 g (76%) of methyl ester *Va*.

Methyl esters VIa *and* VIIa. The aldehyde[1] *II* (3.89 g, 10 mmol) gave 215 mg (5%) of methyl ester *VIa* and 3.20 g (72%) of methyl ester *VIIa*.

Methyl esters VIIIa *and* IXa. The aldehyde[1] *III* (3.89 g, 10 mmol) afforded 175 mg (4%) of methyl ester *VIIIa* and 3.11 g (70%) of methyl ester *IXa*.

General Procedure for Preparation of Hydroxy Derivatives *IVb*, *VIb*, and *VIIIb*

p-Toluenesulfonic acid monohydrate (875 mg, 4.6 mmol) was added to a solution of protected methyl ester (2 mmol) in mixture of benzene (15 ml) and methanol (30 ml). After heating to 45 °C for 3 h, the solvents were evaporated in vacuo, the residue was partitioned between ether and water and the aqueous phase was extracted with ether. The combined organic phases were washed with water, saturated aqueous solution of potassium hydrogen carbonate, and water. The residue was chromatographed on a column of silica gel (60 g). Light petroleum–benzene–ether (50 : 45 : 5) eluted nonpolar impurities; the same solvents in the ratio 40 : 40 : 20 eluted the product.

Methyl (20Z)-3β-hydroxypregna-5,20-diene-21-carboxylate (IVb). The methyl ester *IVa* (885 mg, 2 mmol) afforded 693 mg (97%) of hydroxy derivative *VIb*, m.p. 122 – 125 °C (ether), $[\alpha]_D$ −185° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 608, 3 495 (OH); 1 711 (C=O); 1 641 (C=C). ^{1}H NMR spectrum: 6.17 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 11.5); 5.75 d, 1 H (H-21, *J*(20,21) = 11.5); 5.35 bd, 1 H (H-6, *J* = 4.5); 3.69 s, 3 H ($COOCH_3$); 3.49 m, 1 H (H-3, *W* = 36); 1.01 s, 3 H (3 × H-19); 0.71 s, 3 H (3 × H-18). For $C_{23}H_{34}O_3$ (358.5) calculated: 77.05% C, 9.56 % H; found: 76.88 % C, 9.37 % H.

Methyl (20Z)-3β-hydroxy-5α-pregn-20-en-21-carboxylate (VIb). The methyl ester *VIa* (889 mg, 2 mmol) gave 680 mg (94%) of hydroxy derivative *VIb*, m.p. 141 – 143 °C (ether) $[\alpha]_D$ −106° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 611, 3 495 (OH); 1 717 (C=O); 1 641 (C=C). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5; *J*(20,21) = 11.5); 5.77 d, 1 H (H-21, *J*(20,21) = 11.5); 3.69 s, 3 H ($COOCH_3$); 3.47 m, 1 H (H-3, *W* = 36); 0.81 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{23}H_{36}O_3$ (360.5) calculated: 76.62% C, 10.06% H; found: 76.76% C, 9.92%H.

Methyl (20Z)-3α-hydroxy-5α-pregn-20-en-21-carboxylate (VIIIb). The methyl ester *VIIIa* (889 mg, 2 mmol) afforded 694 mg (96%) of hydroxy derivative *VIIIb*, m.p. 104 – 105 °C (ether–hexane) $[\alpha]_D$ −98° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 616, 3 488 (OH); 1 718 (C=O); 1 640 (C=C). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 12.0); 5.77 d, 1 H (H-21, *J*(20,21) = 12.0); 4.03 m, 1 H (H-3, *W* = 14); 3.69 s, 3 H ($COOCH_3$); 0.78 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{23}H_{36}O_3$ (360.5) calculated: 76.62% C, 10.06% H; found: 76.85% C, 10.31% H.

General Procedure for Preparation of Hemisuccinates *IVd*, *VId*, and *VIIId*

2-(Trimethylsilyl)ethyl hydrogen butanedioate[4] (218 mg, 1.0 mmol) and 4-dimethylaminopyridine (7 mg, 60 mol) were added to a solution of the hydroxy derivative (0.5 mmol) in tetrahydrofuran (4 ml). After addition of 0.5M solution of N,N'-dicyclohexylcarbodiimide in benzene (1.3 ml), the reaction mixture was stirred at room temperature for 6 h, diluted with light petroleum (10 ml) and set aside 10 min. The separated N,N'-dicyclohexylurea was filtered off, washed with light petroleum, the filtrate was taken down in vacuo and the residue was chromatographed on a column of silica gel (25 g). Light petroleum–benzene–ether (50 : 49 : 1) eluted nonpolar impurities, light petroleum–benzene–ether (50 : 48 : 2) washed out the succinate. A solution of succinate in tetrahydrofuran (4 ml) was stirred with 1M solution of tetrabutylamonium fluoride in tetrahydrofuran (1 ml) for 8 h at room temperature. The mixture was diluted with benzene (150 ml) and washed with 10% sulfuric acid (2 times) and water (3 times). Evaporation of the solvent and crystallization of the residue from hexane–dichloromethane (−78 °C) afforded the hemisuccinate.

(20Z)-21-Methoxycarbonylpregna-5,20-diene-3β-yl hydrogen butanedioate (IVd). Hydroxy derivative *IVb* (179 mg, 0.5 mmol) afforded 236 mg of succinate *IVc*. IR spectrum (tetrachloromethane): 1 731

(C=O); 1 642 (C=C); 1 252, 859, 838 (Si–C); 1 171 (C–O). The succinate *IVc* was converted into hemisuccinate *IVd* (160 mg, 70%), m.p. 146 – 149 °C, $[\alpha]_D$ −147° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 716 (C=O); 1 641 (C=C); 1 174 (C–O). ^{1}H NMR spectrum: 6.17 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 12.0); 5.79 d, 1 H (H-21, *J*(20,21) = 12.0); 4.64 m, 1 H (H-3, *W* = 36); 3.69 s, 3 H ($COOCH_3$); 2.64 bs, 4 H ($OOCCH_2CH_2COO$); 1.02 s, 3 H (3 × H-19); 0.70 s, 3 H (3 × H-18). For $C_{27}H_{38}O_6$ (458.6) calculated: 70.72% C, 8.35% H; found: 70.57% C, 8.59% H.

(20Z)-21-Methoxycarbonyl-5α-pregn-20-en-3β-yl hydrogen butanedioate (VId). Hydroxy derivative *VIb* (180 mg, 0.5 mmol) was converted into succinate *VIc* (235 mg). IR spectrum (tetrachloromethane): 1 731 (C=O); 1 641 (C=C); 1 252, 860, 838 (Si–C); 1 174 (C–O). The succinate *VIc* afforded 162 mg (70%) of hemisuccinate *VId*, m.p. 146 – 147 °C, $[\alpha]_D$ −80° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 717 (C=O); 1 640 (C=C); 1 177, 1 003 (C–O). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 12.0); 5.78 d, 1 H (H-21, *J*(20,21) = 12.0); 4.72 m, 1 H (H-3, *W* = 36); 3.69 s, 3 H ($COOCH_3$); 2.62 m, 4 H ($OOCCH_2CH_2COO$); 0.82 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{27}H_{40}O_6$ (460.6) calculated: 70.41% C, 8.75% H; found: 70.71% C, 8.89% H.

(20Z)-21-Methoxycarbonyl-5α-pregn-20-en-3α-yl hydrogen butanedioate (VIIId). Hydroxy derivative *VIIIb* (180 mg, 0.5 mmol) afforded 247 mg of succinate *VIIIc*. IR spectrum (tetrachloromethane): 1 729 (C=O); 1 641 (C=C); 1 252, 859, 832 (Si–C); 1 171 (C–O). The succinate *VIIIc* was converted into hemisuccinate *VIIId* (137 mg, 60%), m.p. 127 – 129 °C, $[\alpha]_D$ −64° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 716 (C=O); 1 640 (C=C); 1 178 (C–O). ^{1}H NMR spectrum: 6.17 dd, 1 H (H-20, *J*(17,20) = 10.5, *J*(20,21) = 12.0); 5.78 d, 1 H (H-21, *J*(20,21) = 12.0); 5.04 m, 1 H (H-3, *W* = 14); 3.69 s, 3 H ($COOCH_3$); 2.67 bs, 4 H ($OOCCH_2CH_2COO$); 0.79 s, 3 H (3 × H-19); 0.68 s, 3 H (3 × H-18). For $C_{27}H_{40}O_6$ (460.6) calculated: 70.41% C, 8.75% H; found: 70.65% C, 9.01% H.

Methyl (20Z)-3β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-pregna-5,20-diene-21-carboxylate (*IVe*)

A dry mixture of hydroxy derivative *IVb* (240 mg, 0.67 mmol), silver silicate[5] (1 g) and ground molecular sieve 4A (500 mg) was stirred in vacuo (10 Pa) 2 h. The flask was then filled with argon under slight overpressure (about 5 kPa) and 1,2-dichloroethane (4 ml) was injected trough a septum. The mixture was stirred at room temperature for 30 min and then a solution of 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl bromide (600 mg, 1.46 mmol) in 1,2-dichloroethane (2 ml) was added through the septum. After stirring at room temperature for 20 h, the catalyst was filtered through a column of Celite. The column was washed with chloroform, and the combined organic phases were washed with 5% aqueous potassium hydrogen carbonate solution and water. The solvents were evaporated, the residue was chromatographed on a column of silica gel (40 g) in benzene–ethyl acetate (100 : 1 – 10 : 1). Yield 347 mg (75%) of product *IVe*, m.p. 162 – 164 °C (ethanol), $[\alpha]_D$ −113° (*c* 0.2, chloroform). IR spectrum (chloroform): 1 756 (C=O, acetate); 1 720 (C=O, ester); 1 641 (C=C); 1 039 (C–O, acetate). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, *J*(17,20) = 10.5; *J*(20,21) = 11.5); 5.79 d, 1 H (H-21, *J*(20,21) = 11.5); 5.23 t, 1 H (H-3', *J*(3',2') ≈ *J*(3',4') ≈ 9); 5.06 t, 1 H (H-4', *J*(4',3') ≈ *J*(4',5') ≈ 9); 4.96 dd, 1 H (H-2', *J*(2',1') = 7.4; *J*(2',3') ≈ 9); 4.59 d, 1 H (H-1', *J*(1',2') = 7.4); 4.28 dd, 1 H (H-6'a, *J*(6'a,6'b) = 12.4; *J*(6'a,5') = 4.9); 4.09 dd, 1 H (H-6'b, *J*(6'b,6'a) = 12.4; *J*(6'b,5') = 2.7); 3.80 – 3.60 m, 2H (H-5'and H-3); 3.69 s, 3 H ($COOCH_3$); 2.07 s, 2.02 s, 2.01 s, 2.00 s, 3 × 3 H (3 × CH_3COO); 0.99 s, 3 H (3 × H-19); 0.70 s, 3 H (3 × H-18). For $C_{37}H_{52}O_{12}$ (688.8) calculated: 64.52% C, 7.61% H; found: 64.67% C, 7.59% H.

Methyl (20Z)-3β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-5α-pregn-20-en-21-carboxylate (*VIe*)

Title compound *VIe* was prepared from hydroxy derivative *VIb* (200 mg, 0.56 mmol), 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (600 mg, 1.46 mmol), and corresponding amount of further reagents analogously as described in the preceding preparation. Yield 300 mg (78%) of product *VIe*, m.p. 168 – 169 °C (ethanol), $[\alpha]_D$ −66° (*c* 0.2, chloroform). IR spectrum (chloroform): 1 756 (C=O, acetate); 1 720 (C=O,

ester); 1 641 (C=C); 1 039 (C–O, acetate). ^{1}H NMR spectrum: 6.17 dd, 1 H (H-20, J(17,20) = 10.5; J(20,21) = 11.5); 5.77 d, 1 H (H-21, J(20,21) = 11.5); 5.23 t, 1 H (H-3', J(3',2') ≈ J(3',4') ≈ 9); 5.05 t, 1 H (H-4', J(4',3') ≈ J(4',5') ≈ 9); 4.96 dd, 1 H (H-2', J(2',1') = 7.5; J(2',3') ≈ 9); 4.59 d, 1 H (H-1', J(1',2') = 7.5); 4.28 dd, 1 H (H-6'a, J(6'a,6'b) = 12.0; J(6'a,5') = 4.9); 4.09 dd, 1 H (H-6'b, J(6'b,6'a) = 12.0; J(6'b,5') = 2.7); 3.67 s, 3 H ($COOCH_3$); 3.65 m, 1 H (H-5', W = 20); 2.07 s, 2.02 s, 2.01 s, 2.00 s, 4 × 3 H (4 × CH_3COO); 0.78 s, 3 H (3 × H-19); 0.65 s, 3 H (3 × H-18). For $C_{37}H_{54}O_{12}$ (690.8) calculated: 64.33% C, 7.88% H; found: 64.47% C, 7.91% H.

Methyl (20*Z*)-3α-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-5α-pregn-20-en-21-carboxylate (*VIIIe*)

Title compound *VIIIe* was prepared from hydroxy derivative *VIIIb* (250 mg, 0.69 mmol), 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (600 mg, 1.46 mmol), and corresponding amount of further reagents analogously as described above. Yield 340 mg (71%) of product *VIIIe*, m.p. 148 – 150 °C (ethanol), $[\alpha]_D$ −70° (*c* 0.2, chloroform). IR spectrum (chloroform): 1 756 (C=O, acetate); 1 720 (C=O, ester); 1 641 (C=C); 1 039 (C–O, acetate). ^{1}H NMR spectrum: 6.16 dd, 1 H (H-20, J(17,20) = 10.4; J(20,21) = 11.2); 5.78 d, 1 H (H-21, J(20,21) = 11.4); 5.24 t, 1 H (H-3', J(3',2') ≈ J(3',4') ≈ 9); 5.07 t, 1 H (H-4', J(4',3') ≈ J(4',5') ≈ 9); 5.00 dd, 1 H (H-2', J(2',1') = 7.7; J(2',3') ≈ 9); 4.55 d, 1 H (H-1', J(1',2') = 7.7); 4.27 dd, 1 H (H-6'a, J(6'a,6'b) = 12.5); J(6'a,5') = 5.0); 4.10 dd, 1 H (H-6'b, J(6'b,6'a) = 12.5; J(6'b,5') = 3.0); 3.93 m, 1 H (H-3, W = 13); 3.69 s, 3 H ($COOCH_3$); 3.67 m, 1 H (H-5', W = 20); 2.07 s, 2.04 s, 2 × 3 H (2 × CH_3COO); 2.02 s, 6 H (2 × CH_3COO); 0.75 s, 3 H (3 × H-19); 0.66 s, 3 H (3 × H-18). For $C_{37}H_{54}O_{12}$ (690.8) calculated: 64.33% C, 7.88% H; found: 64.50% C, 7.90% H.

Methyl (20*Z*)-3β-(β-D-Glucopyranosyloxy)pregna-5,20-diene-21-carboxylate (*IVf*)

To a solution of acetate *IVe* (290 mg, 0.42 mmol) in mixture of benzene (5 ml) and methanol (10 ml) 1M sodium methoxide in methanol (0.1 ml) was added . The mixture was allowed to stand at room temperature for 24 h and neutralized with solid carbon dioxide (about 300 mg). After evaporation, the residue was chromatographed on a column of silica gel (40 g) in chloroform–methanol (20 : 1 – 10 : 1). Crystallization from methanol afforded 175 mg (80%) of glucoside *IVf*, m.p. 245 – 253°C (decomposition), $[\alpha]_D$ −158° (*c* 0.2, methanol). IR spectrum (KBr): 3 404 (OH); 1 721 (C=O); 1 648 (C=C ester); 1 192, 1 171 (C–O ester); 1 075, 1 034, 1 020 (C–O). For $C_{29}H_{44}O_8$ (520.7) calculated: 67.01% C, 8.58% H; found: 67.27% C, 8.62% H.

Methyl (20*Z*)-3β-(β-D-Glucopyranosyloxy)-5α-pregn-20-en-21-carboxylate (*VIf*)

The title compound was prepared from acetate *VIe* (240 mg, 0.34 mmol) analogously as described in the preceding preparation; yield 149 mg (82%) of glucoside *VIf*, m.p. 248 – 252 °C (decomposition), $[\alpha]_D$ −99° (*c* 0.2, methanol). IR spectrum (KBr): 3 403 (OH); 1 722 (C=O); 1 652 (C=C); 1 172 (C–O ester); 1 074, 1 033 (C–O). For $C_{29}H_{46}O_8$ (522.7) calculated: 66.64% C, 8.87% H; found: 66.70% C, 8.89% H.

Methyl (20*Z*)-3α-(β-D-Glucopyranosyloxy)-5α-pregn-20-en-21-carboxylate (*VIIIf*)

The title compound was prepared from acetate *VIIIe* (200 mg, 0.29 mmol) analogously as described above; yield 124 mg (82%) of amorphous glucoside *VIf*. $[\alpha]_D$ −60° (*c* 0.2, methanol). IR spectrum (KBr): 3 425 (OH); 1 727 (C=O); 1 641 (C=C); 1 171 (C–O ester); 1 075, 1 027 (C–O). For $C_{29}H_{46}O_8$ (522.7) calculated: 66.64% C, 8.87% H; found: 66.67% C, 8.91% H.

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