STEROIDS WITH THE β-CROTONATE (2-BUTENOATE) SIDE CHAIN*

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Ethyl (20*E*)-3β-methoxymethoxy-24-nor-5,20(22)-choladien-23-oate (*Va*) and analogous derivatives with 5α,5β and $\Delta^{5,14}$ steroid moiety (*Vb*,*Vc* and *Vd*, respectively) were prepared by Wittig–Horner reaction of the corresponding ketones IIa—IId with diethyl ethoxycarbonylmethylphosphonate. In this case the reaction affords exclusively the (*E*)-isomers, in contrast with the Peterson reaction with lithium salt of ethyl 2-(trimethylsilyl)acetate which gives a mixture of (*E*)- and (*Z*)-isomers at the $\Delta^{20(22)}$ double bond. The structure of the products was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy. The crotonates *Va*—*Vd* were further converted into the 3-O-succinyl derivatives *VIIIa*—*VIIId*.

Steroidal crotonates with the digitoxigenin-like skeleton[1] belong to compounds interesting with respect to the study of structural requirements of cardiotonic activity. They were prepared by Reformatski reaction[1] or by addition of lithium ethoxyacetylide[1–3] to 3β,14-dihydroxy-5β,14β-pregnan-20-one derivatives; however, both syntheses are complicated and lead to mixtures of stereoisomers which can be separated only with difficulty. In connection with synthesis of the β-crotonate side chain in model systems we set out to seek other methods and to check their stereoselectivity.

For the preliminary experiments we chose 3β-hydroxy-5-pregnen-20-one (*Ia*) protected as the methoxymethoxy derivative *IIa* (ref.[4]). Peterson reaction[5,6] of *IIa* with lithium salt of ethyl 2-(trimethylsilyl)acetate afforded in 47% yield the condensation product which, as shown by the ^{1}H NMR spectrum, was a mixture of the (*Z*)- and (*E*)-isomers (*IVa* and *Va*). According to the $C_{(18)}$—H signal intensities (δ 0·68 and 0·59, respectively), the (*Z*) : (*E*) ratio was 1 : 4. The mixture behaved as a single product in thin-layer chromatography and its crystallization from ethanol practically did not change the isomer ratio. Analytical samples of both the isomers *IVa* and *Va* were obtained by repeated chromatography on silica gel. The ^{1}H NMR spectra of both isomers differ mainly in chemical shifts of the $C_{(18)}$—H, $C_{(21)}$—H

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and $C_{(22)}$—H signals (Table I). The $C_{(21)}$—H proton shifts for the isomers *IVa* and *Va* (δ 1·90 and 2·18, respectively) are practically identical with the values published[7] for β-alkylcrotonates in general (δ 1·88 and 2·15, respectively). The dif-

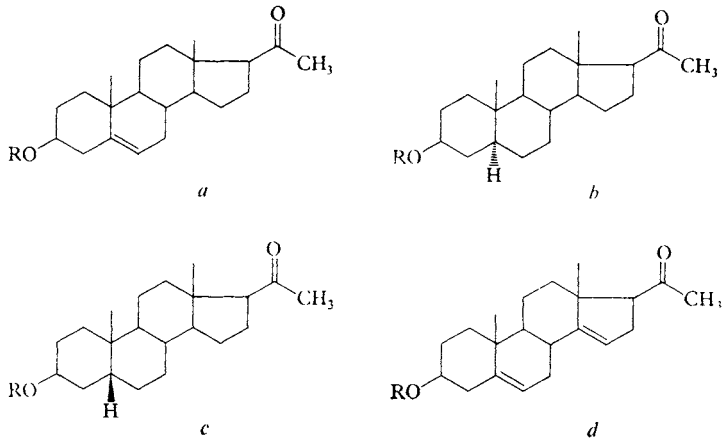
TABLE I

^{1}H NMR Spectral data for the ketones *II* and the crotonates *IV*– *VIII*

Compound[a]	$C_{(18)}$—H_3	$C_{(19)}$—H_3	$C_{(3)}$—H	$C_{(6)}$—H	$C_{(21)}$—H_3	$C_{(22)}$—H
IIa[b,c]	0·63 s	1·01 s	[d]	5·36 m	2·11 s	—
IIb[c]	0·59 s	0·79 s	[d]	[d]	2·08 s	—
IIc[c]	0·60 s	0·95 s	3·87 m	[d]	2·09 s	—
IId[c]	0·86 s	1·00 s	[d]	5·38 m	2·13 s	—
IVa[c,e]	0·68 s	1·01 s	3·42 m	5·36 m	1·90 d	5·79 dq
Va[c,e]	0·59 s	1·00 s	3·41 m	5·34 m	2·18 d[f]	5·68 p[f]
Vb[c,e]	0·57 s	0·81 s	3·50 m	[d]	2·17 d[f]	5·68 p[f]
Vc[c,e]	0·57 s	0·95 s	3·90 m	[d]	2·17 d[f]	5·68 p[f]
Vd[c,e]	0·81 s	1·02 s	3·44 m	5·41 m	2·21 d[f]	5·76 p[f]
VIa[c]	0·58 s	0·99·s	3·48 m	5·33 bd[g]	2·18 d[f]	5·69 bs
VIb[c]	0·57 s	0·96 s	3·57 m	[d]	2·17 d[f]	5·68 bs
VIc[c]	0·55 s	0·94 s	4·08 m	[d]	2·15 d[f]	5·68 m
VId[c]	0·80 s	1·02 s	3·48 m	5·40 m	2·20 d[f]	5·77 bs
VIIa[c]	0·58 s	1·00 s	4·62 m	5·35 bd[g]	2·18 d[f]	5·68 bs
VIIb[c]	0·56 s	0·98 s	[d]	[d]	2·16 d[f]	5·66 bs
VIIc[c]	0·55 s	0·95 s	5·12 m	[d]	2·15 d[f]	5·68 bs
VIId[c]	0·78 s	1·00 s	[d]	5·41 bd[g]	2·19 d[f]	5·74 bs
VIIIa[c]	0·58 s	1·00 s	4·62 m	5·37 bd	2·17 d[f]	5·69 bs
VIIIb[c,e,h]	0·61 s	0·86 s	4·68 m	[d]	2·15 bs	5·69 bs
VIIIc[c,e,h]	0·61 s	0·99 s	5·09 m	[d]	2·16 d[f]	5·69 p[f]
VIIId[c]	0·81 s	1·03 s	4·64 m	5·44 bd[g]	2·21 d[f]	5·75 bs

[a] For conditions of the measurements see Experimental; unless stated otherwise, 60 MHz spectral parameters; [b] ref.[4]; [c] other signals: *IIa*–*IId*, *IVa* and *Va*–*Vd*: 3·34–3·38 s and 4·63–4·69 s (OCH_2OCH_3), *IId*: 5·02 m ($C_{(15)}$—H), *Vd*–*VIIId*: 5·23 m ($C_{(15)}$—H), *IVa*, *V*–*VIII*(*a*–*d*): 1·25–1·28 t and 4·12–4·15 q, J = 7·2 ($COOCH_2CH_3$), *VIIa*–*VIId*: 2·70–2·73 m ($OOCCH_2$.CH_2COO) and 4·73–4·75 s ($COOCH_2CCl_3$), *VIIIa*–*VIIId*: 2·57–2·63 bs ($OOCCH_2CH_2$.COO); [d] undeterminable value; [e] 200 MHz spectral parameters; [f] $J_{21,22}$ = 1·2; [g] J = 4·5; [h] measured in C^2HCl_3–$C^2H_3O^2H$ (1 : 1).

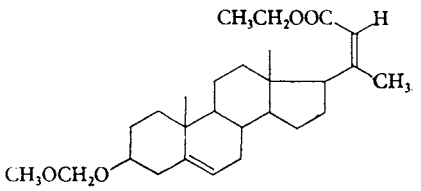
ference in the chemical shifts is caused mainly by the anisotropic shielding effect of the carbonyl which can exist only in the (*E*)-isomer, but its extent may vary case to case (*cf.* the 5β,14β-hydroxy derivative[1]). Independently, we have proved the configuration of the crotonate double bond by measuring the Overhauser effect



a *b* *c* *d*

I, R = H
II, R = CH_2OCH_3
III, R = $COCH_3$

between the 21-methyl and $C_{(22)}$—H signals. For the (*Z*)-isomer *IVa* ($C_{(21)}$—H_3 and $C_{(22)}$—H *cis*) irradiation of the $C_{(21)}$—H signal resulted in the NOE enrichment of (+19·8 ± 0·9)% whereas for the (*E*)-isomer the effect was negligible (−1·8 ± 1)%. The effect on the $C_{(18)}$—H signal can be ascribed to a different orientation of the crotonate double bond relative to the steroid skeleton in the (*Z*)- and (*E*)-isomers. A similar shift was observed[8,9] also for $\Delta^{20(22)}$-unsaturated derivatives devoid of the carboxyl in position 23. Configuration of the β-alkylcrotonate double bond in isoprenoid derivatives has been successfully assigned by ^{13}C NMR spectroscopy[10] (methyl signal for (*E*)-isomers: δ 16–19, for (*Z*)-isomers δ 31–33). However, the spectra of our derivatives *IVa* and *Va* (Table II) show a less marked difference (for $C_{(21)}$ $\delta_{(E)}$ 20·7, $\delta_{(Z)}$ 24·0) and both values are outside the cited intervals. In addition to the $C_{(21)}$ and $C_{(22)}$ signals, the $C_{(17)}$ signal is significantly affected, too; a smaller effect was found for the $C_{(12)}$, $C_{(13)}$ and $C_{(18)}$ signals. Also the coupling constants ^3J (^{13}C—^1H) are sensitive to configurational changes at trisubstituted double bonds[11] but they are difficult to determine in spectra of complex derivatives because of overlapping of closely spaced signals in the proton coupled spectra. We succeeded to determine the constant ^3J ($C_{(21)}$—$H_{(22)}$) for the (*E*)-isomer *Va*: it amounts to 8 Hz and can be used for comparison with analogous constants in the steroidal crotonates prepared below.



IVa

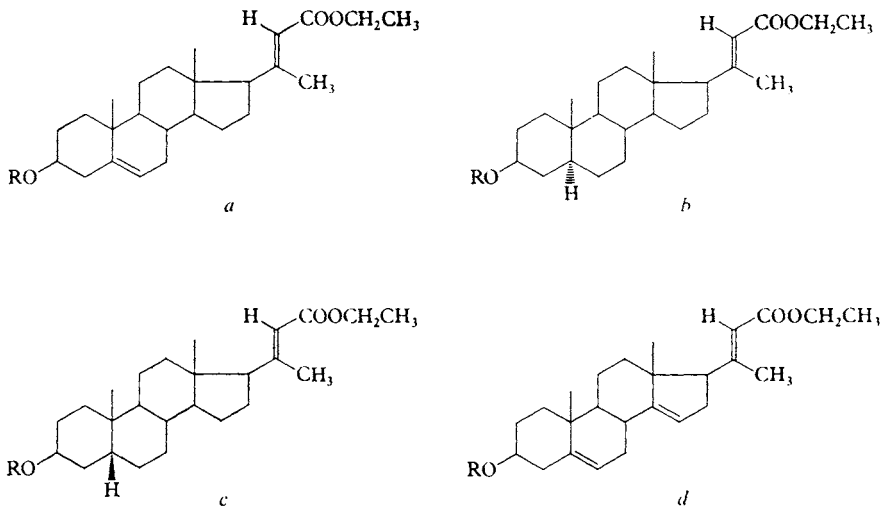
Another applicable preparative approach to unsaturated esters is the Wittig–Horner reaction. Although the corresponding reagent, diethyl ethoxycarbonylmethylphosphonate, reacts with steroidal aldehydes and some ketones[12], no reaction is reported[13] with 20-keto derivatives (pregnenolone acetate *IIIa*). Since the similarly unreactive 17-ketones react successfully at elevated temperature[14], we treated the ketone *IIa* with the reagent in boiling 1,2-dimethoxyethane. Although the reaction was sluggish even under these conditions, after 30 h most of the starting compound reacted and the ester *Va* was isolated in 46% yield. Surprisingly, no detectable amount of the (Z)-isomer *IVa* was formed, according to the ^{1}H NMR spectrum of the product.

The methoxymethoxy derivative *Va* was deblocked in a benzene–ethanol mixture to give the hydroxy derivative *VIa*; we used ethanol instead of the commonly used methanol to eliminate possible reesterification. The ultraviolet spectrum of the derivative *VIa* exhibits a maximum at 228 nm and corresponds to the spectra of analogous unsaturated steroidal esters (226–230 nm, ref.[1]; 232 nm, ref.[2]).

For the biological activity tests the hydroxy derivative *VIa* was converted to the hemisuccinate *VIIIa*. Because of good previous results, we used the indirect method[15] consisting in reaction of the alcohol with 2,2,2-trichloroethyl hemisuccinate in dichloromethane using N,N'-dicyclohexylcarbodiimide as condensation reagent. The product was deblocked with zinc and acetic acid in aqueous tetrahydrofuran. The whole conversion *VIa*→*VIIa*→*VIIIa* gave the compound *VIIIa* in 60% yield.

To establish a basis for comparison of chemical and biological properties of steroidal crotonates containing various steroid skeletons, we applied the above-mentioned reaction sequence to other pregnan-20-one derivatives differing in the mode of ring annelation and/or the presence of a double bond at the junction of the rings A and B or (in the last case) C and D. According to the above scheme, 3β-hydroxy-5α-pregnan-20-one (*Ib*) was converted by the sequence *Ib*→*IIb*→*Vb*→*VIb*→ →*VIIb*→*VIIIb* into the hemisuccinate belonging to the 5α-series and, analogously, 3β-hydroxy-5β-pregnan-20-one (*Ic*) afforded the hemisuccinate *VIIIc* as the representative of the 5β-series. We prepared also the $\Delta^{5,14}$ ester *VIIId* according to the scheme *Id*→*IId*→*Vd*→*VId*→*VIId*→*VIIId*.

Since in all these syntheses the condensation of the ketone with the phosphonate salt represents the key step, we tried to optimize this reaction. It seems that neither prolonged reaction time nor excess of the reagents affect significantly the yield; the



V, R = CH_2OCH_3
VI, R = H
VII, R = $OCCH_2CH_2COOCH_2CCl_3$
VIII, R = $OCCH_2CH_2COOH$

best results were obtained when the phosphonate salt was prepared separately and added portionwise to the reaction mixture (see preparation of the derivative *Vc*). In all the studied series the reaction with the phosphonate is stereoselective, giving exclusively the (*E*)-isomers *Va* – *Vd*.

Configuration at the $\Delta^{20(22)}$ double bond in *Vb* – *Vd* has been proved by comparison of ^1H and ^{13}C NMR spectra (Table I and II, respectively) with those of the derivative *Va*. The shifts of the $C_{(18)}$—H, $C_{(21)}$—H and $C_{(22)}$—H protons in compounds *Va* – *Vc* are practically identical; the values for *Vd* correspond to the published data[1] for the 5β,Δ^{14}-derivative (δ 0·82, 2·18, and 5·75, respectively). Similar agreement has been found in the ^{13}C NMR spectra for the $C_{(17)}$, $C_{(21)}$ and $C_{(22)}$ signals of *Va* – *Vd*; the compounds *Va* – *Vc* have identical shifts also for the $C_{(12)}$, $C_{(13)}$ and $C_{(18)}$ signals. The coupling constants ^3J ($C_{(21)}$—$H_{(22)}$) obtained for *Vb* (8·4 Hz) and *Vc* (7·9 Hz) correspond to that for *Va* (8 Hz). The carbon signals in the spectra of derivatives *Va* – *Vd* were assigned using the attached proton test spectra[16], signal multiplicities in the proton-coupled spectra, and comparison with the published values for compounds *Ia* (ref.[17]), *Ib* and *Ic* (ref.[18]) and the measured values for *IIa* and *Id* (Table II).

The prepared steroidal crotonates with established (*E*)-configuration of the side-chain double bond are designed as models for testing structural requirements for the cardiotonic activity since an analogous derivative containing the natural cardiotonic skeleton exhibits such activity[1].

EXPERIMENTAL

Melting points were determined on a Boetius micromelting point apparatus (GDR). Optical rotations were taken on a Perkin–Elmer 141 MC polarimeter and are given in 0·01 deg kg^{-1} m^2. IR Spectra were taken on a UR-20 (Zeiss, Jena) spectrophotometer, wavenumbers are given in cm^{-1}. NMR Spectra were measured on CW Tesla BS-467 (60 MHz for ^1H) or FT Varian XL-200 (200·058 MHz for ^1H and 50·309 MHz for ^{13}C nuclei) instruments in deuteriochloroform, with tetramethylsilane as internal standard, chemical shifts are given on δ-scale (ppm), coupling constants (J) and band widths (W) in Hz. All parameters were obtained by first-order analysis. Mass spectra were measured on an AEI-901 spectrometer. Preparative chromatography was carried out on columns of silica gel (according to Pitra, 60—120 μm, Service Laboratories

TABLE II

^{13}C NMR Spectral data for steroidal crotonates *IVa*, *Va*—*Vd* and ketones *Id* and *IIa*

Carbon[a]	*IVa*	*Va*	*Vb*	*Vc*	*Vd*	*Id*	*IIa*
$C_{(1)}$	37·3	37·3	37·0	30·5	37·0	36·9	37·3
$C_{(2)}$	29·0	28·9	28·7	25·3	28·8	30·8	28·9
$C_{(3)}$	76·9	76·9	76·2	72·1	76·8	70·9	76·8
$C_{(4)}$	39·6	39·6	35·2	31·3	39·4	41·0	39·5
$C_{(5)}$	140·9	140·8	44·9	37·1	139·9	140·0	140·7
$C_{(6)}$	121·7	121·5	28·7	26·2	121·3	120·6	121·4
$C_{(7)}$	32·0	31·8	32·0	26·7	29·7	29·4	31·8
$C_{(8)}$	32·0	32·2	35·7	36·0	31·2	30·7	31·8
$C_{(9)}$	50·3	50·3	54·4	40·1	50·0	50·0	50·0
$C_{(10)}$	36·7	36·8	35·6	35·0	37·0	36·7	36·7
$C_{(11)}$	20·8	21·0	21·2	21·1	21·7	21·4	21·1
$C_{(12)}$	36·9	38·6	38·7	39·0	41·0	41·5	38·8
$C_{(13)}$	47·1	44·5	44·7	44·9	48·6	48·0	44·0
$C_{(14)}$	55·9	56·7	56·4	56·6	152·8	151·0	56·9
$C_{(15)}$	24·7	24·3	24·2	24·2	118·0	117·7	24·5
$C_{(16)}$	25·0	25·0	24·9	25·0	32·9	30·9	22·8
$C_{(17)}$	49·8	60·4	60·4	60·6	62·2	65·0	63·7
$C_{(18)}$	14·2	13·0	13·1	13·1	17·9	17·8	13·2
$C_{(19)}$	19·4	19·4	12·2	23·9	19·1	18·7	19·3
$C_{(20)}$	159·9	159·9	160·0	160·1	159·7	210·3	209·3
$C_{(21)}$	24·0	20·7[b]	20·6[c]	20·7[d]	20·4	30·8	31·5
$C_{(22)}$	119·0	116·0	115·9	115·9	116·3	—	—
$C_{(23)}$	166·7	167·0	166·9	167·0	166·9	—	—
OCH_2O	94·7	94·7	94·5	94·5	94·7	—	94·7
OCH_3	55·2	55·2	55·0	55·1	55·1	—	55·1
OCH_2CH_3	59·4	59·4	59·3	59·4	59·5	—	—
OCH_2CH_3	14·4	14·4	14·3	14·3	14·3	—	—

[a] For conditions of measurement see Experimental; [b] ^3J ($C_{(21)}$—$H_{(22)}$) = 8 Hz; [c] ^3J ($C_{(21)}$—$H_{(22)}$) = 8·3 Hz; [d] ^3J ($C_{(21)}$—$H_{(22)}$) = 7·9 Hz.

of this Institute), thin-layer chromatography (TLC) was performed on silica gel G according to Stahl (Woelm). Spots were detected by spraying with sulfuric acid followed by heating. For HPLC a stainless steel column (500 × 12·6 mm) packed with Separon Si (10 μm, Laboratory Instruments, Czechoslovakia) was used. Solutions were dried over anhydrous sodium sulfate and taken down on a rotatory evaporator at bath temperature 40—50°C and pressure 2—2·5 kPa. Analytical samples were dried over phosphorus pentoxide at about 25 Pa.

Peterson Olefination of Ketone *IIa*

A solution of 1-butyllithium in hexane (10·4 ml, *c* 1·6 mol l^{-1}) was added under argon at −78°C to a stirred solution of diisopropylamine (1·68 g; 16·6 mmol) in tetrahydrofuran (83 ml). The mixture was stirred for 30 min at −78°C and ethyl 2-(trimethylsilyl)acetate (ref.[19]; 2·67 g; 16·6 mmol) was added. After stirring for 10 min at −78°C, a solution of the ketone *IIa* (ref.[4], 2 g; 5·5 mmol) in tetrahydrofuran (15 ml) was added and the stirring was continued for 1 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. The residue was chromatographed on a column of silica gel (200 g) in light petroleum–benzene–ether (50 : 50 : 1). The first two fractions (A : 860 mg, B : 260 mg) afforded 1·12 g (47%) of a mixture of the unsaturated esters *IVa* and *Va*. Further elution with light petroleum–benzene–ether (50 : 50 : 2) recovered the ketone *IIa* (937 mg; 47%). The fraction A (860 mg) was crystallized from hot ethanol and the product (495 mg) was chromatographed on silica gel (20 μ, 350 × 25 mm column, benzene–ether 100 : 1). Purification of the first chromatographic fractions by HPLC (benzene–ether 100 : 1) gave an analytical sample of the (*Z*)-isomer *IVa*, m.p. 142—143°C (ethanol); $[\alpha]_D$ −163° (*c* 0·14; chloroform). IR spectrum (tetrachloromethane): 1 715, 1 629 (conjugated ester), 1 153, 1 107, 1 049 (C—O—C). For $C_{27}H_{42}O_4$ (430·6) calculated: 75·31% C, 9·83% H; found: 75·24% C, 9·83% H. Fraction B (260 mg) of the original mixture was chromatographed on silica gel (20 μ, 350 × 25 mm column, benzene–ether 100 : 1) affording 40 mg of the (*E*)-isomer *Va*, identical with the sample prepared in the subsequent experiment.

Ethyl (20*E*)-3β-Methoxymethoxy-24-nor-5,20(22)-choladien-23-oate (*Va*)

Diethyl ethoxycarbonylmethylphosphonate (7·93 ml; 40 mmol) was added during 10 min to a stirred suspension of sodium hydride (960 mg; 40 mmol) in 1,2-dimethoxyethane (50 ml) in an argon atmosphere. Stirring was continued for 30 min at room temperature and a solution of the ketone *IIa* (3·6 g; 10 mmol) in 1,2-dimethoxyethane (35 ml) was added. After stirring at 85°C for 30 h, the mixture was evaporated *in vacuo* and the residue was partitioned between ether and water. The aqueous layer was extracted with ether, the combined organic portions were taken down *in vacuo* and the residue was chromatographed on a column of alumina (100 g) in benzene–ether (9 : 1). The crude product (4·2 g) was chromatographed on a silica gel column (500 g) in light petroleum–benzene–ether (49 : 49 : 2), affording 1·97 g (46%) of the ester, *Va*, m.p. 99—102°C (light petroleum); $[\alpha]_D$ −46° (*c* 0·4; chloroform). IR spectrum (tetrachloromethane): 1 706, 1 634 (unsaturated ester), 1 150, 1 103, 1 042 (C—O—C). Mass spectrum (*m/z*): M^+ 430. For $C_{27}H_{42}O_4$ (430·6) calculated: 75·31% C, 9·83% H; found: 75·03% C, 9·80% H.

Ethyl (20*E*)-3β-Hydroxy-24-nor-5,20(22)-choladien-23-oate (*VIa*)

A mixture of the ester *Va* (1·97 g: 4·57 mmol), *p*-toluenesulfonic acid monohydrate (1·97 g; 10·36 mmol), benzene (40 ml) and ethanol (100 ml) was kept at 40°C for 11 h. After evaporation of the solvents *in vacuo*, the residue was partitioned between ether and water. The aqueous layer was extracted with ether and the combined organic phases were washed with aqueous potassium

hydrogen carbonate and water. Evaporation *in vacuo* afforded 1·75g (99%) of the hydroxy derivative *VIIa*. An analytical sample was crystallized from light petroleum–ether, m.p. 132–134°C: $[\alpha]_D$ −55° (*c* 0·3; chloroform). IR spectrum (tetrachloromethane): 3 623, 3 410 (OH), 1 712, 1 640 (unsaturated ester); UV spectrum (ethanol): log ε 4·23 (228 nm). For $C_{25}H_{38}O_3$ (386·6) calculated: 77·68% C, 9·91% H; found: 77·38% C, 10·11% H.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5,20(22)-choladien-3β-yl 3-[4-(2,2,2-Trichloroethoxy)--4-oxobutanoate] (*VIIa*)

A mixture of the hydroxy derivative *VIa* (386 mg; 1 mmol), benzene (15 ml), 4-(2,2,2-trichloroethoxy)-4-oxobutanoic acid[20] (470 mg; 1·88 mmol), N,N′-dicyclohexylcarbodiimide (220 mg; 1·07 mmol) and 4-dimethylaminopyridine (5 mg) was stirred at room temperature for 8 h. After dilution with light petroleum (20 ml), the separated N,N′-dicyclohexylurea was filtered off and the solvents were evaporated *in vacuo*. The residue was subjected to chromatography on a column of silica gel (60 g) in light petroleum–ether (90 : 10) to give 478 mg (77%) of the ester *VIIa*, m.p. 116–118°C (light petroleum–ether); $[\alpha]_D$ −24° (*c* 0·25; chloroform). IR spectrum (tetrachloromethane): 1 715, 1 640 (unsaturated ester), 1 761, 1 735 (—$OOCCH_2CH_2COOCH_2CCl_3$). For $C_{31}H_{43}Cl_3O_6$ (618·0) calculated: 60·25% C, 7·01% H, 17·21% Cl; found: 60·00% C, 6·91% H, 17·19% Cl.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5,20(22)-choladien-3β-yl 3-(3-Carboxypropanoate) (*VIIIa*)

A mixture of the ester *VIIa* (412 mg; 0·67 mmol), tetrahydrofuran (14 ml), acetic acid (14 ml), water (1·4 ml) and zinc (140 mg) was stirred at 0°C for 4 h. During this time, further three 140 mg portions of zinc were added. The mixture was filtered through Celite and the filtrate was mixed with toluene (30 ml) and taken down. The residue was coevaporated with toluene (30 ml) and chromatographed on a column of silica gel (50 g) in dichloromethane–methanol (95:5) to yield 250 mg (77%) of the hemisuccinate *VIIIa*, m.p. 129–132°C (ethanol–water); $[\alpha]_D$ −44° (*c* 0·2; chloroform). IR spectrum (chloroform): 1 714, 1 635 (unsaturated ester), 1 714, 1 730 sh (COOR); 2 500–3 500 (COOH). For $C_{29}H_{42}O_6$ (486·7) calculated: 71·58% C, 8·70% H; found: 71·76% C, 8·97% H.

3β-Methoxymethoxy-5α-pregnan-20-one (*IIb*)

N,N-Dimethylaniline (2·53 ml; 20 mmol), followed by chloromethyl methyl ether (1·52 ml; 20 mmol), was added to a solution of the hydroxy derivative *Ib* (3·16 g; 10 mmol) in dichloromethane (50 ml). After stirring at room temperature for 20 h, the mixture was diluted with ether (500 ml), washed with dilute hydrochloric acid, water, aqueous potassium hydrogen carbonate and again with water. The solvents were evaporated *in vacuo*, the residue was dissolved in a mixture of benzene–ether (1 : 1) and filtered through a column of alumina (50 g) which was then washed with the same solvent system. Yield 3·59 g (99%) of the oily product which crystallized on standing; m.p. 66–68°C; $[\alpha]_D$ +82° (*c* 0·3; chloroform). IR spectrum (tetrachloromethane): 1 746 (C=O), 1 150, 1 107, 1 047 (C—O—C). For $C_{23}H_{38}O_3$ (362·6) calculated: 76·20% C, 10·56% H; found: 75·92% C, 10·53% H.

Ethyl (20*E*)-3β-Methoxymethoxy-24-nor-5α-chol-20(22)-en-23-oate (*Vb*)

Diethyl ethoxycarbonylmethylphosphonate (11·4 ml; 57·5 mmol) was added during 10 min in an argon atmosphere to a suspension of sodium hydride (1·38 g; 57·5 mmol) in 1,2-dimethoxyethane (60 ml). After stirring for 30 min at room temperature, a solution of the ketone *IIb*

(3·47 g; 9·6 mmol) in 1,2-dimethoxyethane (20 ml) was added, the mixture was stirred at 85°C for 24 h, taken down *in vacuo* and the residue was partitioned between ether and water. The aqueous layer was extracted with ether and the combined organic extracts were taken down *in vacuo*. The residue was subjected to column chromatography on alumina (100 g) in benzene–ether (9 : 1) and the eluted crude product (4·5 g) was rechromatographed on silica gel (500 g) in light petroleum–benzene–ether (49 : 49 : 2), affording 1·31 g (32%) of the ester *Vb*, m.p. 91 to 93°C (light petroleum); $[\alpha]_D$ +9° (*c* 0·25; chloroform). IR spectrum (tetrachloromethane): 1 715, 1 639 (unsaturated ester), 1 147, 1 106, 1 045 (C—O—C). Mass spectrum (*m/z*): M^+ 432. For $C_{27}H_{44}O_4$ (432·6) calculated: 74·96% C, 10·25% H; found: 74·66% C, 10·46% H.

Ethyl (20*E*)-3β-Hydroxy-24-nor-5α-chol-20(22)-en-23-oate (*VIb*)

A mixture of the ester *Vb* (1 g; 2·31 mmol), benzene (20 ml), ethanol (50 ml) and *p*-toluenesulfonic acid monohydrate (1 g; 5·26 mmol) was warmed to 40°C for 15 h and evaporated *in vacuo*. The residue was partitioned between ether and water and the aqueous layer was extracted with ether. The combined organic solutions were washed with aqueous potassium hydrogen carbonate and water and taken down. Chromatography of the residue on a column of silica gel (50 g) in light petroleum–benzene–ether (45 : 45 : 10) afforded 637 mg (71%) of the hydroxy derivative *VIb*; m.p. 148—150°C (ether); $[\alpha]_D$ +15° (*c* 0·2; chloroform). IR spectrum (chloroform): 1 705, 1 635 (unsaturated ester), 3 612, 3 408 (OH). UV spectrum (ethanol): log ε 4·30 (227 nm). For $C_{25}H_{40}O_3$ (388·6) calculated: 77·27% C, 10·38% H; found: 77·03% C, 10·27 %.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5α-chol-20(22)-en-3β-yl 3-(4-(2,2,2-Trichloroethoxy)-4-oxobutanoate) (*VIIb*)

4-(2,2,2-Trichloroethoxy)-4-oxobutanoic acid[20] (334 mg; 1·34 mmol), N,N′-dicyclohexylcarbodiimide (160 mg; 0·77 mmol) and 4-dimethylaminopyridine (5 mg) were added to a solution of the hydroxy derivative *VIb* (275 mg; 0·71 mmol) in benzene (15 ml). After stirring for 7 h at room temperature, the mixture was diluted with light petroleum (20 ml), the precipitated N,N′-dicylohexylurea was filtered and the solvents were evaporated in vacuo. Chromatography on a silica gel column (30 g) in light petroleum–ether (90 : 10) gave 320 mg (73%) of the ester *VIIb*, m.p. 92—95°C (light petroleum): $[\alpha]_D$ 0 (*c* 0·2; chloroform). IR spectrum (tetrachloromethane): 1 718, 1 638 (unsaturated ester), 1 763, 1 735 ($OOCCH_2CH_2COOCH_2CCl_3$). For $C_{31}H_{45}Cl_3O_6$ (620·1) calculated: 60·05% C, 7·32% H, 17·15% Cl; found: 60·23% C, 7·59% H, 17·29% Cl.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5α-chol-20(22)-en-3β-yl 3-(3-Carboxypropanoate) (*VIIIb*)

A mixture of the ester *VIIb* (266 mg; 0·43 mmol), tetrahydrofuran (9 ml), acetic acid (9 ml), water (0·9 ml) and zinc (90 mg) was stirred at 0°C for 5 h. During this time four additional 90 mg portions of zinc were added. The mixture was filtered through Celite, the filtrate was mixed with toluene (25 ml) and taken down *in vacuo*. The residue was coevaporated with toluene (25 ml) and chromatographed on a column of silica gel (15 g) in chloroform–methanol (90 : 10) to give the crude product (180 mg). Dissolution in hot ethanol and precipitation on addition of water and cooling yielded 130 mg (62%) of the amorphous hemisuccinate *VIIIb*, $[\alpha]_D$ +8° (*c* 1·2; chloroform–methanol 1 : 1). IR spectrum (KBr): 1 716, 1 637 (unsaturated ester), 1 730 sh (saturated ester), 3 500—2 500 (COOH). For $C_{29}H_{44}O_6$ (488·7) calculated: 71·28% C, 9·08% H; found: 71·61% C, 9·01% H.

3β-Methoxymethoxy-5β-pregnan-20-one (*IIc*)

A mixture of the hydroxy derivative *Ic* (2·75 g; 8·63 mmol), dichloromethane (40 ml), N,N-dimethylaniline (1·45 ml; 11·44 mmol) and chloromethyl methyl ether (0·87 ml; 11·45 mmol) was stirred at room temperature 8 h. The same amount of the reagents was then added and the stirring was continued for 12 h. The mixture was diluted with dichloromethane (150 ml) and poured into water (200 ml). The organic layer was washed with 5% hydrochloric acid (2 × 200 ml) and saturated solution of potassium hydrogen carbonate (1 × 200 ml), dried, concentrated and filtered through a column of alumina (50 ml). The column was washed with dichloromethane and the combined eluates were taken down, leaving 3·0 g (96%) of the oily derivative *IIc*; $[\alpha]_D$ +92° (*c* 0·2; chloroform). IR spectrum: 1 706, 1 356 (CH_3CO), 1 148, 1 102, 1 049 (C—O—C). For $C_{23}H_{38}O_3$ (362·6) calculated: 76·20% C, 10·56% H; found: 75·92% C, 10·53% H.

Ethyl (20*E*)-3β-Methoxymethoxy-24-nor-5β-chol-20(22)-en-23-oate (*Vc*)

Diethyl ethoxycarbonylmethylphosphonate (1·59 ml; 8 mmol) was added under argon to a suspension of sodium hydride (192 mg; 8 mmol) in 1,2-dimethoxyethane (5 ml). The mixture was stirred at room temperature for 20 min and the thus-obtained solution of the phosphonate salt was added dropwise at 50°C under argon during 30 min to a solution of the methoxymethoxy derivative *IIc* (1·45 g; 4·0 mmol) in 1,2-dimethoxyethane (10 ml). Stirring at 50°C was continued for 10 h and another portion of the salt, made from the same amounts of hydride and diethyl ethoxycarbonylmethylphosphonate, was added. After stirring for 8 h the addition was repeated. After further 6 h (24 h total) of stirring at 50°C the mixture was cooled and taken down. The residue was coevaporated with benzene, dissolved in ether, washed with saturated sodium chloride solution (2×), dried and concentrated to a minimum volume. After filtration through a column of alumina (100 g) and washing the column with benzene–ether (10 : 1), the filtrate was evaporated and the residue dried affording 1·69 g of an oil which was chromatographed on silica gel (100 g) in benzene–ether (100 : 1). The chromatography furnished 1·38 g (80%) of the oily ester *Vc* and 200 mg (12%) of the recovered ketone *IIc*. The ester *Vc* had $[\alpha]_D$ +21° (*c* 2·2; chloroform). IR spectrum (tetrachloromethane): 1 716, 1 638, 1 219 (unsaturated ester), 1 101, 1 049, 1 149 (C—O—C). For $C_{27}H_{44}O_4$ (432·7) calculated: 74·96% C, 10·25% H; found: 75·16% C, 10·09% H.

Ethyl (20*E*)-3β-Hydroxy-24-nor-5β-chol-20(22)-en-23-oate (*VIc*)

A solution of the methoxymethoxy derivative *Vc* (1·3 g; 3·0 mmol) in benzene (50 ml) was mixed with ethanol (50 ml) and conc. hydrochloric acid (0·5 ml). After stirring at 40°C for 12 h, the mixture was taken down, the residue coevaporated with benzene (3 × 20 ml), dissolved in dichloromethane and applied on a column of alumina (10 g). The product was eluted with chloroform and after evaporation of the solvent crystallized from warm ethanol; yield 890 mg (79%) of the ester *VIc*, m.p. 131—133°C; $[\alpha]_D$ +21° (*c* 0·2; chloroform). IR spectrum (tetrachloromethane): 1 714, 1 640 (unsaturated ester), 3 625 (OH). UV spectrum (ethanol): log ε 4·19 (228 nm). For $C_{24}H_{40}O_3$ (376·6) calculated: 76·55% C, 10·71% H; found: 76·75% C, 10·54% H.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5β-chol-20(22-)-en-3β-yl 3-(4-(2,2,2-Trichloroethoxy)--4-oxobutanoate) (*VIIc*)

To a solution of the hydroxy derivative *VIc* (200 mg; 0·51 mmol) in benzene (11 ml) were added 4-(2,2,2-trichloroethoxy)-4-oxobutanoic acid[20] (243 mg; 0·97 mmol), N,N′-dicyclohexylcarbodiimide (116 mg; 0·56 mmol) and 4-dimethylaminopyridine (4 mg) and the mixture was stirred at room temperature for 8 h. After dilution with light petroleum (25 ml), the separated N,N′-dicyclohexylurea was filtered and the solvents were evaporated *in vacuo*. Chromatography on a co-

lumn of silica gel (30 g) in light petroleum–ether (92 : 8) afforded 225 mg (71%) of the oily ester *VIIc*; $[\alpha]_D$ +18° (*c* 1·2; chloroform). IR spectrum (tetrachloromethane): 1 719, 1 639 (unsaturated ester), 1 762, 1 733 ($OOCCH_2CH_2COOCH_2CCl_3$). For $C_{31}H_{45}Cl_3O_6$ (620·1) calculated: 60·05% C, 7·32% H, 17·15% Cl; found: 59·81% C, 7·37% H, 17·35% Cl.

(20*E*)-22-Ethoxycarbonyl-23,24-dinor-5β-chol-20(22)-en-3β-yl 3-(3-Carboxypropanoate) (*VIIIc*)

A mixture of the ester *VIIc* (200 mg; 0·32 mol), tetrahydrofuran (7 ml), acetic acid (7 ml), water (0·7 ml) and zinc (70 mg) was stirred at 0°C for 5 h. Four 70 mg portions of zinc were added during this time. The mixture was filtered through Celite, the filtrate was mixed with toluene (20 ml) and evaporated *in vacuo*. The residue was coevaporated with toluene (25 ml) and chromatographed on a column of silica gel (15 g) in chloroform–methanol (9 : 1). The obtained crude product (150 mg) was dissolved in hot ethanol and precipitated by addition of water and cooling; yield 107 mg (68%) of the amorphous hemisuccinate *VIIIc*; $[\alpha]_D$ +23° (*c* 1·2; chloroform–methanol 1 : 1). IR spectrum (KBr): 1 713, 1 636 (unsaturated ester), 1 735 (saturated ester), 3 500—2 500 (COOH). For $C_{29}H_{44}O_6$ (488·7) calculated: 71·28% C, 9·08% H; found: 71·49% C, 9·41% H.

3β-Methoxymethoxy-5,14-pregnadien-20-one (*IId*)

A mixture of the hydroxy derivative *Id* (refs[21–23]; 0·8 g; 2·5 mmol), dichloromethane (15 ml), N,N-dimethylaniline (1 ml; 7·9 mmol) and chloromethyl methyl ether (0·4 ml: 5·3 mmol) was stirred at room temperature for 16 h and processed as described for the derivative *IIb*. Crystallization from dichloromethane–pentane afforded 640 mg (70%) of the derivative *IId*, m.p. 138 to 140°C, $[\alpha]_D$ −11° (*c* 0·25; chloroform). IR spectrum (tetrachloromethane): 3 055 (C=C—H), 1 710, 1 358 (CH_3CO), 1 152, 1 111, 1 048 (C—O—C). For $C_{23}H_{34}O_3$ (358·5) calculated: 77·05% C, 9·56% H; found: 76·78% C, 9·52% H.

Ethyl (20*E*)-3β-Methoxymethoxy-24-nor-5,14,20(22)-cholatrien-23-oate (*Vd*)

The solution of the phosphonate salt was prepared as described in the preparation of *Vc* from sodium hydride (240 mg; 10·1 mmol), 1,2-dimethoxyethane (10 ml) and diethyl ethoxycarbonylmethylphosphonate (2 ml; 10·1 mmol). One third (about 3 ml) of the salt solution was added at 50°C under argon to the derivative *IId* (550 mg; 1·53 mmol) in 1,2-dimethoxyethane (5 ml) and the mixture was stirred at 80°C for 8 h. The second third was added, the stirring was continued for further 10 h and the remaining portion was added. After stirring for 6 h, the mixture was worked up as described for the derivative *Vc*. Chromatography on silica gel in benzene–ether (100 : 1) afforded 490 mg (75%) of the product fraction which was crystallized from dichloromethane–ethanol to give 260 mg (40%) of the ester *Vd*, m.p. 133—134°C, $[\alpha]_D$ −73° (*c* 0·35; chloroform). IR spectrum (tetrachloromethane): 1 716, 1 640 (unsaturated ester), 1 151, 1 111 1 049 (C—O—C). Mass spectrum (*m/z*): 428 (M^+), 413 (M−15), 396 (M−32), 383 (M−45), 366 (M−62). For $C_{27}H_{40}O_4$ (428·6) calculated: 75·66% C, 9·41% H; found: 75·76% C, 9·62% H. In addition, 100 mg (18%) of the ketone *IId* was recovered.

Ethyl (20*E*)-3β-Hydroxy-24-norchola-5,14,20(22)-trien-23-oate (*VId*)

The methoxymethoxy derivative *Vd* (250 mg; 0·58 mmol) was deblocked in a mixture of benzene (10 ml), ethanol (10 ml) and hydrochloric acid (0·1 ml) in the same manner as described for the derivative *Va*, affording 210 mg (94%) of *VId*, m.p. 152—155°C (dichloromethane–ethanol): $[\alpha]_D$ −77° (*c* 0·33; chloroform). IR spectrum (tetrachloromethane): 3 625, 1 053 (OH), 1 715,

1 640 (unsaturated ester), 1 676 (C=C). UV spectrum (ethanol): log ε 4·27 (225 nm). For C_{25}·.$H_{36}O_3$ (384·6) calculated: 78·08% C, 9·44% H; found: 78·18% C, 9·43% H.

(20*E*)-22-Ethoxycarbonyl-23,24-dinorchola-5,14,20(22)-trien-3β-yl 3-(4-(2,2,2-Trichloroethoxy)-4-oxobutanoate) (*VIId*)

To a solution of the hydroxy derivative *VId* (60 mg; 0·16 mmol) in benzene (2 ml) were added 4-(2,2,2-trichloroethoxy)-4-oxobutanoic acid[20] (75 mg; 0·3 mmol), N,N'-dicyclohexylcarbodiimide (35 mg; 0·17 mmol), 4-dimethylaminopyridine (1 mg) and the mixture was stirred at room temperature for 8 h. After dilution with light petroleum (4 ml), the separated N,N'-dicyclohexylurea was filtered and the filtrate was taken down *in vacuo*. Chromatography of the residue on a column of silica gel (10 g) in light petroleum–ether (95 : 5) gave 70 mg (73%) of the ester *VIId*, m.p. 112–114°C (light petroleum–ether), $[\alpha]_D$ −42° (*c* 1·0; chloroform). IR spectrum (tetrachloromethane): 1 718, 1 640 (unsaturated ester); 1 763, 1 739 ($OOCCH_2CH_2COOCH_2CCl_3$). For $C_{31}H_{41}Cl_3O_6$ (616·0) calculated: 60·44% C, 6·71% H, 17·27% Cl; found: 60·74% C, 6·66% H, 17·61% Cl.

(20*E*)-22-Ethoxycarbonyl-23,24-dinorchola-5,14,20(22)-trien-3β-yl 3-(3-Carboxypropanoate) (*VIIId*)

A mixture of the ester *VIId* (62 mg; 0·1 mmol), tetrahydrofuran (2 ml), acetic acid (2 ml), water (0·2 ml) and zinc (20 mg) was stirred at 0°C for 5 h. During this time, four 20 mg portions of zinc were added. The mixture was filtered through Celite, the filtrate mixed with toluene (10 ml) and taken down *in vacuo*. After coevaporation with toluene (10 ml), the residue was chromatographed on a column of silica gel (10 g) in chloroform–methanol (9 : 1) to give 40 mg of crude product. Crystallization from light petroleum–dichloromethane afforded 30 mg (62%) of the hemisuccinate *VIIId*, m.p. 165–168°C, $[\alpha]_D$ −53° (*c* 0·7; chloroform). IR spectrum (KBr): 1 713, 1 638 (unsaturated ester), 1 732 (saturated ester), 3 500–2 500 (COOH). For $C_{29}H_{40}O_6$ (484·6) calculated: 71·87% C, 8·32% H; found: 71·74% C, 8·53% H.

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