USE OF MALONIC ACID DERIVATIVES FOR THE CONSTRUCTION OF SUBSTITUTED SIDE CHAINS IN THE POSITION 17β OF STEROIDS*

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Using the Knoevenagel reaction condensation products of 3β-acetoxyandrost-5-en-17-one with diethyl malonate, ethyl cyanoacetate and malononitrile were prepared which were converted to derivatives of 3β-hydroxypregn-5-en-21-oic acid. Reductions and oxidations of the double bonds in the positions 5,6 and 17,20 and the stereochemistry at atoms $C_{(17)}$ and $C_{(20)}$ of some derivatives were also investigated. From derivatives of 23,24-dinorchola-5,17(20)-diene-21,22-diol compounds with a 1,3-dioxane ring in the side chain were prepared.

In connection with our studies concerning steroids with substituted side chains[1-5] and partial syntheses of cardenolides[6], a synthetic approach to the derivatives with substituted branched side chain was elaborated, based on Knoevenagel condensation of 3β-acetoxyandrost-5-en-17-one (*I*) with diethyl malonate, catalysed with titanium(IV) chloride[7], or with ethyl cyanoacetate, catalysed with ammonium acetate[8], or with malononitrile, catalysed with β-alanine[9].

In the condensation of *I* with diethyl malonate according to ref.[7] the yield dependend on the reaction conditions and was about 20%. The product *II* was purified by chromatography on silica gel. It was characterized by IR spectrometry (a band at 1 636 cm^{-1} corresponding to the double bond in the neighbourhood of the ester groups and the bands at 1 730, 1 235 and 1 037 cm^{-1} corresponding to the ester groups vicinal to the double bond), mass spectrum (M^+ 472) and ^{1}H-NMR spectrum as well as further methods. Condensation of *I* with ethyl cyanoacetate under catalysis with ammonium acetate was carried out according to literature[8], giving rise to ester nitrile *VII*. The reaction of steroidal ketones with malononitrile under catalysis with β-alanine is known from patent literature in a modification where malononitrile is used as the reaction medium[9]. It seems suitable to carry out the condensation

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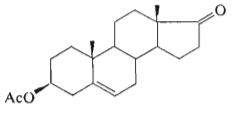
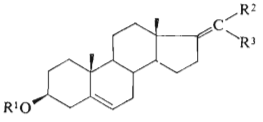
of *I* with malononitrile similarly as in the preceding case, *i.e.* in benzene as the reaction medium. In this modification the yields are higher and the product is purer.

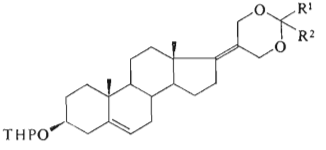
Diester *II* was deacetylated to *III*. It was found that the use of sodium methoxide in methanol is more advantageous than the use of potassium carbonate or potassium hydroxide. The yield of the deacetylated product *III* is quantitative. Hydroxy compound *III* was protected in the position $C_{(3)}$ by reaction with dihydropyran in dichloromethane under catalysis with *p*-toluenesulfonic acid. After recrystallisation from pentane the yield of derivative *IV* was 56%. The blocking of the 3β-hydroxy group by the tetrahydropyranyl group was confirmed by ^{1}H-NMR spectrometry (signals of hydrogens of the tetrahydropyranyloxy group at 3·52 and 4·73 ppm) and other physical methods.



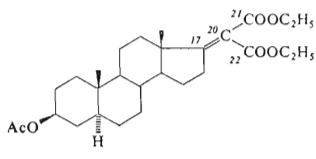
I



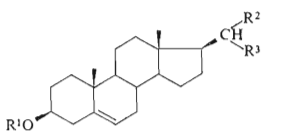
II, R^1 = Ac, $R^2 = R^3 = COOC_2H_5$
III, R^1 = H, $R^2 = R^3 = COOC_2H_5$
IV, R^1 = THP, $R^2 = R^3 = COOC_2H_5$
V, R^1 = THP, $R^2 = R^3 = CH_2OH$
VI, R^1 = H, $R^2 = R^3 = CH_2OH$
VII, R^1 = Ac, R^2; $R^3 = COOC_2H_5$; CN
VIII, R^1 = Ac, $R^2 = R^3$ = CN
IX, $R^1 = R^2$ = H, R^3 = COOH
X, $R^1 = R^2$ = H, $R^3 = COOCH_3$



XI, $R^1 + R^2 = =O$
XII, R^1; R^2 = H; C_6H_5



XX



XIII, R^1 = H, $R^2 = R^3 = COOC_2H_5$
XIV, R^1 = Ac, $R^2 = R^3 = COOC_2H_5$
XV, R^1 = Ac, R^2; $R^3 = COOC_2H_5$; CN
XVI, R^1 = Ac, $R^2 = R^3$ = CN
XVII, R^1 = THP, $R^2 = R^3 = COOCH_3$
XVIII, R^1 = H, $R^2 = R^3 = COOCH_3$
XIX, R^1 = H, $R^2 = R^3 = CH_2OH$

In the reduction of diester *IV* with sodium dihydro-bis(2-methoxyethoxy)aluminate (synhydride) only the ester group is selectively reduced and both double bonds in the molecule remain intact. The structure of diol *V*, formed in this reduction in a 81% yield, was proved by ^{13}C-NMR spectrometry, mass spectrometry (m/z 412 = M − H_2O) and further methods. Diol *V* was condensed with benzaldehyde in benzene in the presence of anhydrous copper(II) sulfate to benzylidene derivative *XII* in 39% yield. The yield was low, owing to repeated crystallizations which are necessary for the elimination of the residues of benzaldehyde. Similarly, diol *V* was submitted to the reaction with 1,1'-carbonyldiimidazole in dimethylformamide[10], giving rise to cyclic carbonate *XI* in 53% yield. Both heterocyclic derivatives *XI* and *XII*, derived from 5-alkylidene-1,3-dioxane, were studied and characterized by spectral methods, especially ^{1}H-NMR spectrometry. In the case of benzylidene derivative *XII* two singlets of isomeric protons in the position $C_{(2')}$ of the dioxane ring were found in a 5 : 8 ratio of their integrals, indicating the ratio of both diastereoisomers (at 5·63 and 5·57 ppm in δ-scale).

During the reduction of the unsaturated diester arrangement of compound *II* with lithium aluminum hydride in tetrahydrofuran at room temperature reduction of the double bond and of the ester group took place, under formation of a mixture of an unsaturated and saturated diol *VI* and *XIX*, shown by mass spectrometry and ^{13}C NMR spectrometry. From the ratio of signals in the ^{13}C-NMR spectrum it follows that the ratio of *VI* and *XIX* was about 4 : 1.

The reduction with sodium borohydride also showed some specificity. In this reduction the 17,20-double bond was reduced preferentially and the ester group remained intact, or – depending on the medium – was partially hydrolysed. The reduction of diester *II* took place very slowly and with difficulty. We were unable to modify the reaction conditions so that the resulting mixture did not contain unsaturated diester *II* in addition to the reduction product *XIV* and the reduction product with subsequent hydrolysis *XIII*. Hydrolysis may be prevented by carrying out the reaction in the presence of molecular sieves of 0·4 nm porosity.

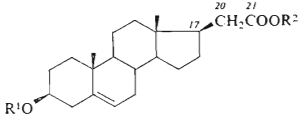
In contrast to this, the reduction of derivatives *VII* and *VIII* with sodium borohydride took place relatively easily and in high yield. Both reduced derivatives *XV* and *XVI* were characterized mainly by ^{1}H-NMR spectrometry: in both spectra the signal at 2·9 ppm belonging to the hydrogens on $C_{(16)}$ ppm is absent. In the spectrum of the cyano ester *XV* two doublets were observed, at 3·42 (J = 8 Hz) and 3·28 (J = 10 Hz) ppm, in a 4 : 1 ratio of their integrals, corresponding to both $C_{(20)}$ diastereoisomers. Similarly, ester nitrile *VII* may be reduced to the saturated derivative *XV* with calcium borohydride. Dinitrile *XVI* was characterized similarly as *XV*, assuming that the doublet at 3·58 ppm belonged to the hydrogen on $C_{(20)}$. Both derivatives, *XV* and *XVI*, were hydrolysed under simultaneous decarboxylation in boiling ethylene glycol with potassium hydroxide to the acid *XXI*, described earlier[11]. This acid was characterized by conversion to the methyl ester *XXII* the

physical constants of which fully agree with the literature data[12]. Thus, the β-configuration on $C_{(17)}$ in derivatives *XV* and *XVI* may be considered proved.

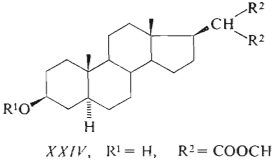
Hydrogenation of diester *II* on palladium on charcoal gave derivative *XX* the 17,20-double bond of which was not attacked even in an alternative experiment when Adams catalyst was used.

In order to obtain derivatives with an analogous but saturated side chain the condensation of the protected ester *XXIII* was used (ref.[12]) which was converted to its lithium salt with methyl chloroformate (for the procedure see ref.[13]). Thus diester *XVII* was obtained in a high yield. The tetrahydropyranyl protecting group was split off using *p*-toluenesulfonic acid. The unsaturated hydroxy derivative *XVIII* obtained was hydrogenated on Adams catalyst, giving rise to the saturated derivative *XXIV*. The structures of compounds *XVII*, *XVIII* and *XXIV* were demonstrated by ^{1}H-NMR spectrometry.

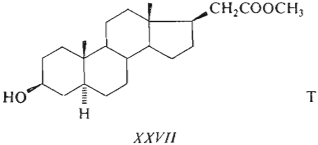
Using hydrolysis combined with decarboxylation of unsaturated diester *II*, carried out in ethylene glycol and in the presence of potassium hydroxide unsaturated hydroxy acid *IX* was obtained which was converted to the methyl ester *X*. From a comparison of the ^{1}H-NMR spectrum of *X* with the literature data[14], especially the values of the chemical shifts of $C_{(20)}$ hydrogen atoms and hydrogen atoms of the methyl group on $C_{(13)}$, it follows that the substance *X* obtained is pure 17(20)*E* isomer. Its hydrogenation on Adams catalyst gave the saturated ester *XXVII* which is also formed on hydrogenation of the unsaturated ester *XXII*. Under the same conditions the 17,20-double bond of the unsaturated ester *II* or *XX* was not reduced.



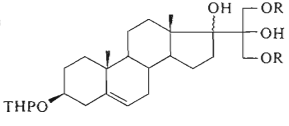
XXI, $R^1 = R^2 = H$
XXII, $R^1 = H$, $R^2 = CH_3$
XXIII, $R^1 = THP$, $R^2 = CH_3$



XXIV, $R^1 = H$, $R^2 = COOCH_3$
XXV, $R^1 = H$, $R^2 = CH_2OH$
XXVI, $R^1 = Ac$, $R^2 = CH_2OAc$



XXVII



XXVIII, R = H
XXIX, R = Ac

This different reactivity of derivatives *II*, *XX* and *X* is probably due to different steric hindrance of the access of hydrogen to the 17,20-double bond. This is supported by the statement from literature[12] that in compounds of the type *X* the 17,20-double bond may be hydrogenated selectively, while the 5,6-double bond cannot. Alcohol *XXV* was prepared on reduction of diester *XXIV*. It was characterized as acetyl derivative *XXVI*.

On oxidation of diol *V* with osmium tetroxide tetrol *XXVIII* was formed, with a non-determined configuration at $C_{(17)}$. However, it may be assumed that the attack on the 17,20-double bond takes place from the α-side of the steroidal skeleton and therefore the bond 17,20 of the side chain of derivative *XXVIII* must be β. Except for the fact that spectral analysis did not show that a mixture of isomers is involved the stereochemistry was not further investigated. Under the conditions used the 5,6-double bond was not oxidized. Owing to solubility, the tetrol was converted to diacetyl derivative *XXIX* which was characterized. Application of acetylation with acetic anhydride in pyridine led to the acetylation of only the unhindered primary hydroxyl groups on $C_{(21)}$ and $C_{(22)}$, as confirmed by ^{1}H-NMR spectrum.

As regards the use of malonic acid derivatives for the construction of substituted side chains in the position 17β of steroids, the methods using the ester nitrile *VII* and dinitrile *VIII* seem preparatively advantageous. The use of diester *II* for the same purpose is much more complex.

EXPERIMENTAL

The melting points were measured on a Boetius (GDR) melting point microscope. The UV spectra were measured on a Specord (GDR) instrument and the IR spectra on a UR-20 Carl Zeiss Jena spectrophotometer (wave-numbers given in cm^{-1}). The ^{1}H-NMR spectra were measured on a Tesla B 467 (60 MHz) instrument and the ^{13}C-NMR spectra on a Jeol FX-60 apparatus. Chemical shifts are given in ppm, δ-scale, and the coupling constants and the band widths in Hz. All values were obtained by first order analysis. The mass spectra were measured on a AEI MS 901 spectrometer. Specific rotations were measured on an Opton Germany type VDRNA instrument, in chloroform, unless stated otherwise. Analytical samples were dried at 13 Pa. Column chromatography was carried out on silica gel according to Pitra (particle size 60—120 μm), prepared in the Service laboratory, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences. Thin-layer chromatography was done on silica gel G according to Stahl (Woelm). The solutions were evaporated under reduced pressure (2 kPa) at 20—40°C after previous drying over magnesium sulfate.

Diethyl 3β-acetoxy-23,24-dinorchola-5,17(20)-diene-21,22-dioate (*II*)

The preparation of *II* was carried out according a procedure from literature[7], using 8·65 g (25 mmol) of ketone *I*. At the end of the preparation chromatography on silica gel (500 g) with hexane as eluent was used. The residue of the main fraction was crystallized from acetone–hexane, affording 2·3 g (20%) of *II*, m.p. 156—159°C, $[\alpha]_D^{25}$ −14·1° (*c* 0·5, measured on a Perkin–Elmer 141 MC instrument). IR spectrum (chloroform): 1 730, 1 235, 1 037 (CH_3COO, esters vicinal to the double bond), 1 636 (double bond vicinal to the ester groups). ^{1}H-NMR spectrum:

5·38 m (1 H, $C_{(6)}$—H), 4·60 m (1 H, $C_{(3)}$—H), 4·26 q and 4·20 q (2 × 2 H, $J = 7·5$ 2 × × OCH_2CH_3), 2·92 m (2 H, $C_{(16)}$—H_2), 2·35 bd (2 H, $J \approx 8$ $C_{(7)}$—H_2), 2·03 s (3 H, CH_3COO), 1·33 t and 1·26 t (2 × 3 H, $J = 7·5$ 2 × —OCH_2CH_3), 1·02 s and 0·99 s (2 × 3 H, angular methyls). ^{13}C-NMR spectrum, observable peaks: 172·57 s, 170·5 s, 167·1 s, 164·5 s, 139·7 s, 122·15 d, 119·02 s, 73·8 d, 60·95 t, 60·55 t, 54·7 d, 49·5 d, 47·17 s, 38·1, 39·9, 36·5, 33·9, 32·2, 31·4, 27·8, 24·04, 21·4, 21·2, 19·2, 16·4, 14·03. Mass spectrum (*m/z*): 472 M^+, 412 (M—CH_3COOH), 366 (M—CH_3COOH—C_2H_5OH), 351 (366—CH_3). For $C_{28}H_{40}O_6$ (472·6) calculated: 71·16% C, 8·53% H; found: 71·07% C, 8·53% H.

Diethyl 3β-hydroxy-23,24-dinorchola-5,17(20)-diene-21,22-dioate (*III*)

Derivative *II* (1 g, 2·1 mmol) was suspended in methanol (50 ml) and 0·1M sodium methoxide solution in methanol (2 ml) was added. The mixture was allowed to stand, under stirring, at room temperature for 8 h and then poured onto ice–water mixture (750 ml). When all the ice had melted the solid material was filtered off and dried. The hydroxy derivative obtained was amorphous, yield 950 mg (87%). IR spectrum (chloroform): 3 610, 1 040 (OH), 1 670 (C=C), 1 726, 1 629, 1 254, 1 032 (esters vicinal to the double bond). Mass spectrum (*m/z*): 430 (M^+), 415 (M—CH_3), 369 ($C_{23}H_{29}O_4$), 270 ($C_{19}H_{26}O$). For $C_{26}H_{38}O_5$ (430·6) calculated: 72·53% C, 8·90% H; found: 72·23% C, 9·10% H.

Diethyl 3β-(2-tetrahydropyranyloxy)-23,24-dinorchola-5,17(20)diene-21,22-dioate (*IV*)

Hydroxy derivative *III* (980 mg, 2·09 mmol) was suspended in dichloromethane (30 ml) and dihydropyran (0·23 ml) and *p*-toluenesulfonic acid (3 mg) were added. After 12 h stirring additional dihydropyran (0·23 ml, *i.e.* a total of 0·46 ml, 6 mmol) and *p*-toluenesulfonic acid (3 mg) were added. After 4 h stirring at room temperature the mixture was extracted with saturated sodium carbonate solution (3 × 100 ml) and the organic layer was dried and evaporated. The residue was crystallized from pentane (30 ml). The crystals were filtered and washed with three 20 ml portions of pentane and dried. Yield, 600 mg (56%) of derivative *IV*, m.p. 150—151°C, $[\alpha]_D^{25}$ +11·5° (*c* 0·31, measured on a Perkin–Elmer 141 MC instrument). IR spectrum (tetrachloromethane): 1 725, 1 232, 1 635 (ROOC—C=C). ^{1}H-NMR spectrum (measured on Varian XL-100 instrument): 5·38 m (1 H, $C_{(6)}$—H), 4·73 m (1 H, —O—CH—O— from tetrahydropyranyl), 4·24 m (4 H, 2 × —OCH_2CH_3), 3·89 m (1 H, $C_{(3)}$—H), 3·52 m (2 H, —O—CH_2— from tetrahydropyranyl), 2·90 m (2 H, $C_{(16)}$—H_2), 2·36 bd (2 H, $J \approx 8$, $C_{(7)}$—H_2), 1·34 t and 1·27 t (2 × 3 H, $J = 7$, 2 × —OCH_2CH_3), 1·02 s and 1·00 s (2 × 3 H, angular methyls). Mass spectrum: (*m/z*): 430 (M—C_5H_8O), 412 (M—$C_5H_{10}O_2$), 369 ($C_{23}H_{29}O_4$), 270 ($C_{19}H_{26}O$). For $C_{31}H_{46}O_6$ (514·7) calculated: 72·34% C, 9·01% H; found: 72·04% C, 8·70% H.

3β-(2-Tetrahydropyranyloxy)-23,24-dinorchola-5,17(20)-diene-21,22-diol (*V*)

Diester *IV* (514 mg, 1 mmol) was dissolved in tetrahydrofuran (20 ml) and a 65% benzene solution of sodium dihydro-bis(2-methoxyethoxy)aluminate, (Synhydride, 1·5 ml, content 5 mmol of H) was added to it. After 5 h standing at room temperature the mixture was decomposed with water and partitioned between ethyl acetate and water. The organic layers were combined and dried. After evaporation 490 mg of a solid were obtained which was crystallized from ethyl acetate. Yield 350 mg (81%) of diol *V*, m.p. 168—170°C, $[\alpha]_D^{25}$ −47·2 (*c* 0·49, measured on a Perkin–Elmer 141 MC instrument). IR spectrum (chloroform): 1 660 (C=C), 3 610 (OH), 1 135, 1 059, 1 031, 998 (—O—). ^{1}H-NMR spectrum (Varian XL-100): 5·33 m (1 H, $C_{(6)}$—H), 4·69 m (1 H, —O—CH— from tetrahydropyranyl), 4·37 m (4 H, $C_{(21)}$—H_2 and $C_{(22)}$—H_2), 4·32 s

(2 H, OH), 3·89 m (1 H, $C_{(3)}$—H), 3·46 m (2 H, —O—CH_2— from tetrahydropyranyl), 1·00 and 0·90 2 s (2 × 3 H, angular methyl). ^{13}C-NMR spectrum, observable peaks: 151·8 s, 141·1 s, 127·5 s, 121·3 d, 96·9 d, 76·02, 64·19, 62·89, 60·82, 56·01, 49·90, 44·83, 40·28, 38·85, 37·81, 37·42, 37·16, 36·77, 31·84, 31·32, 29·76, 29·24, 28·07, 25·60, 24·30, 21·44, 19·36, 17·67. Mass spectrum (m/z): 412 ($M-H_2O$), 310 ($M-H_2O-C_5H_{10}O_2$), 328 ($412-C_5H_8O$), 85 (C_5H_9O). For $C_{27}H_{42}O_4$ (430·6) calculated: 75·31% C, 9·83% H; found: 74·96% C, 9·68% H.

(*R*/*S*)-21,22-O-Benzylidene-3β-(2-tetrahydropyranyloxy)-23,24-dinorchola-5,17(20)-diene--21,22-diol (*XII*)

Diol *V* (215 mg, 0·5 mmol) in benzene (20 ml) was mixed with benzaldehyde (79·6 mg, 0·07 ml; 1·5 equivalents) and anhydrous copper(II) sulfate (250 mg) and the mixture was shaken at room temperature for 3 days. It was then filtered through a column of alumina (5 × 3 cm) and the column was washed with benzene (50 ml). The filtrate was evaporated and the residue crystallized from hexane and recrystallized from methanol. Yield, 100 mg (39%) of the benzylidene derivative *XII*, m.p. 141–145°C. IR spectrum (chloroform) 703, 3 100, 3 070 (phenyl), 1 670 (C=C) 1 137, 1 118, 1 159, 1 130, 1 079, 913 (—O—). UV spectrum (ethanol): λ_{max} 214 nm, λ_{inflex} 257 nm. ^{1}H-NMR spectrum (Varian XL-200): 7·48 m (5 H, phenyl) 5·63 and 5·57 (total 1 H, ratio of the integrals of the areas of both signals 5 : 8, $C_{(2)}$—H from 1,3-dioxane), 5·09 to 4·33 m (4 H, $C_{(21)}$—H_2 and $C_{(22)}$—H_2); observable signals in a 200 MHz spectrum: 5·09, 5·02, 4·63, 4·60, 4·53, 4·47, 4·39, 4·33 ($C_{(21)}$—H_2 and $C_{(22)}$—H_2)), 4·73 bs (1 H, —O—CH—O—, from tetrahydropyranyl), 3·91 m (1 H, $C_{(3)}$—H), 3·52 m (2 H, —O—CH_2— from tetrahydropyranyl), 1·03 s (6 H, angular methyls). For $C_{34}H_{46}O_4$ (518·7) calculated: 78·72% C, 8·94% H; found: 79·00% C, 8·84% H.

21,22-O-Carbonyl-3β-(2-tetrahydropyranyloxy)-23,24-dinorchola-5,17(20)-diene-21,22-diol (*XI*)

Diol *V* (215 mg, 0·5 mmol) was dissolved in dimethylformamide (10 ml) and 1,1′-carbonyldiimidazole (122 mg, 0·75 mmol) was added to it. After 2 h standing and occasional stirring further 1,1′-carbonyldiimidazole (50 mg, 0·3 mmol) was added and the mixture allowed to stand at room temperature for another 20 h. It was then poured into water (200 ml) and extracted with ether (3 × 50 ml). The organic extracts were combined, dried and evaporated. The residue was chromatographed on silica gel plates (3 plates of 20 × 20 cm dimension), using benzene–ether (2 : 1) for elution. Yield 120 mg (53%) of carbonate *XI* which was crystallized from ether. M.p. 170 to 173°C, $[\alpha]_D^{25}$ −148° (*c* 1·5). IR spectrum (chloroform): 1 756 (cyclic carbonate), 1 685 (C=C), 1 185, 1 135, 1 112, 1 108, 1 030, 979 (—O— from tetrahydropyranyl and carbonate). ^{1}H-NMR spectrum (Varian XL-200): 5·35 bm (1 H, $C_{(6)}$—H), 5·01, 4·7 (total of 4 H, degenerated AB systems $C_{(21)}$—H_2 and $C_{(22)}$—H_2), 4·74 bs (1 H, —O—CH—O— from tetrahydropyranyl), 3·91 m (1 H, $C_{(3)}$—H), 3·51 m (2 H, —O—CH_2— from tetrahydropyranyl), 1·04 and 0·94, 2 s (6 H, angular methyls). For $C_{28}H_{40}O_5$ (456·6) calculated: 73·65% C, 8·83% H; found: 73·41% C, 8·88% H.

Ethyl (20*R*)+(20*S*)-3β-acetoxy-20-cyanopregn-5-en-21-oate (*XV*)

Sodium borohydride (286 mg) was added to a solution of ester nitrile *VII* (5 g, 11·76 mmol; prepared according to ref.[8]) in a mixture of ethanol (36 ml) and tetrahydrofuran (80 ml), cooled at 0°C. After 3 h stirring at 0°C the mixture was poured into water (1 l), acidified with hydrochloric acid and the product was extracted with ether. The extract was washed with dilute 5%

hydrochloric acid, water, saturated potassium hydrogen carbonate solution and water and dried. On evaporation, 4·8 g (96%) of product *XV* were obtained, pure according to TLC analysis. ^{1}H-NMR spectrum (Varian XL-100): 5·37 m (1 H, $C_{(6)}$—H), 4·58 m (1 H, $C_{(3)}$—H), 4·24 q (2 H, —OCH_2— from ester group), 3·42 d (0·8 H, J = 9, $C_{(20)}$—H), 3·28 d (0·2 H, J = 10, $C_{(20)}$—H), 2·33 bd (2 H, J = 8, $C_{(7)}$—H), 2·02 s (3 H, CH_3COO), 1·33 t (3 H, J = 7, CH_3 from ethyl ester), 1·04 s (3 H, $C_{(19)}$—H_3), 0·79 s (3 H, $C_{(18)}$—H_3). Mass spectrum (*m/z*): 367 (M—CH_3COOH). For $C_{26}H_{37}NO_4$ (427·6) calculated: 73·04% C, 8·72% H, 3·28% N; found: 73·24% C, 9·06% H, 3·07% N.

3β-Acetoxy-23,24-dinorchola-5,17(20)-diene-21,22-dinitrile (*VIII*)

β-Alanine (450 mg, 5 mmol) and malononitrile (2 g, 25 mmol) were added to a solution of ketone *I* (3·1 g, 10 mmol) in a mixture of benzene (10 ml) and acetic acid (1 ml) and the mixture was boiled using a device for azeotropic elimination of water for 24 h. The mixture was filtered through a layer of diatomaceous earth prewashed with dichloromethane. The combined filtrates were washed with water and saturated sodium hydrogen carbonate solution, dried and evaporated to a syrup (3·5 g). The syrup was dissolved in dichloromethane, boiled with charcoal and the solution was filtered. After concentration of the filtrate the product *VIII* was crystallized from methanol. Yield, 2·58 g of dinitrile *VIII* (69%), m.p. 150—195°C, under decomposition. $[\alpha]_D^{25}$ −57° (*c* 2·01). IR spectrum (tetrachloromethane): 2 235 (=C—CN), 1 606, 3 040, 3 015 (C=C), 1 740, 1 245, 1 035 (CH_3COO). ^{1}H NMR spectrum: 5·38 m (1 H, $C_{(6)}$—H), 4·57 bs (1 H, $W \approx 40$, $C_{(3)}$—H), 2·83 bd (2 H, $J \approx 7$, $C_{(16)}$—H_2), 2·30 bd (2 H, $J \approx 8$, $C_{(7)}$—H_2), 1·99 s (3 H, CH_3COO—), 1·01 s (6 H, angular methyls). For $C_{24}H_{30}N_2O_2$ (378·5) calculated: 76·16% C, 8·00% H, 7·40% N; found: 75·88% C, 7·83% H, 7·38% N.

3β-Acetoxy-23,24-dinorchol-5-ene-21,22-dinitrile (*XVI*)

Dinitrile *VIII* (1·9 g, 5 mmol) was dissolved in tetrahydrofuran (45 ml), diluted with ethanol (15 ml) and stirred with sodium borohydride (150 mg) at room temperature for 4 h. The excess of the hydride was decomposed by addition of a saturated sodium dihydrogen phosphate solution (1 ml) and the mixture was evaporated. The solid residue was extracted with ether, the extract dried and filtered through a column of aluminum oxide. The residue of the filtrate (1·13 g) was crystallized from acetone–methanol. Yield, 1·02 g (54%) of saturated dinitrile *XVI*, m.p. 198°C, $[\alpha]_D^{25}$ −77° (*c* 0·76). IR spectrum (tetrachloromethane): 2 260 (CN), 1 740, 1 243, 1 034 (CH_3COO), 3 040 (=C—H). ^{1}H-NMR spectrum: 5·36 m (1 H, W = 18, $C_{(6)}$—H), 4·60 m (1 H, $C_{(3)}$—H), 3·58 bd (1 H, $J \approx 9$, $C_{(20)}$—H), 2·33 bd (2 H, $J \approx 8$, $C_{(7)}$—H_2), 2·03 s (3 H, CH_3COO), 1·03 s (3 H, $C_{(19)}$—H_3), 0·78 s (3 H, $C_{(18)}$—H_3). For $C_{24}H_{32}N_2O_2$ (380·5) calculated: 75·75% C, 8·48% H, 7·36% N; found: 76·07% C, 8·51% H, 7·36% N.

3β-Hydroxypregn-5-en-21-oic Acid (*XXI*)

A) Dinitrile *XVI* (570 mg, 1·5 mmol) was dissolved in ethylene glycol (35 ml), potassium hydroxide (1·5 g) was added and the mixture refluxed for 8 h. After cooling it was poured into water (150 ml), acidified with sulfuric acid and extracted with ether. The ethereal extracts were dried, filtered with charcoal and evaporated. Crystallization of the residue from methanol gave 325 mg of acid *XXI* (65%), m.p. 232—233°C, under decomposition, $[\alpha]_D^{25}$ −52° (*c* 0·91, methanol).

B) Potassium hydroxide (12 g) was added to a solution of ester nitrile *XV* (4·8 g, 11 mmol) in ethylene glycol (264 ml) and the mixture was refluxed for 30 h. After pouring into diluted hydrochloric acid (1 : 10, 1 l) the product was extracted with ether and the extract washed with

water. Crystallization of the residue from methanol afforded 2·24 g (60%) of acid *XXI*, m.p. 231—238°C, decomp., $[\alpha]_D^{25}$ −51° (*c* 1·06, methanol). Literature[11] gives m.p. 220—229°C, $[\alpha]_D^{25}$ −50° (*c* 1·21, methanol). IR spectrum (KBr): 3 250 (OH), 1 680, 3 000 broad band (COOH), 3 035 (=C—H).

Methyl 3β-hydroxypregn-5-en-21-oate (*XXII*)

A) Acid *XXI* (200 mg, 0·6 mmol) was mixed with methanol (10 ml) and an excess of a diazomethane solution in ether was added. The mixture was allowed to stand at 0°C overnight, filtered through a column of aluminum oxide (which was prewashed with 100 ml of ethyl acetate) and evaporated. The residue (174 mg, 83%) was crystallized from an acetone–ether mixture. The obtained methyl ester *XXII* had m.p. 132—133°C, $[\alpha]_D^{25}$ −59° (*c* 1·1).

B) Anhydrous potassium carbonate (4·81 g) and dimethyl sulfate (2·2 ml) were added to a solution of acid *XXI* (3·7 g, 11·13 mmol) in acetone (50 ml) and refluxed for 9 h under stirring. After cooling the mixture was diluted with an ether–benzene mixture (1 : 1, 200 ml) and poured onto a column of aluminum oxide (300 g). The column was eluted with ether–benzene 1 : 1 and the filtrates were evaporated. Yield, 3·13 g (81%) of methyl ester *XXII*, m.p. 130—133°C (acetone), $[\alpha]_D^{25}$ −58° (*c* 1·71). Lit.[12] gives m.p. 127—130°C, $[\alpha]_D^{25}$ −60°. ^{1}H-NMR spectrum: 5·33 m (1 H, $C_{(6)}$—H), 3·63 s ($COOCH_3$), 3·50 m (1 H, $C_{(3)}$—H), 1·02 and 0·61, 2 s (2 × 3 H, angular methyls).

Dimethyl 3β-Hydroxy-23,24-dinor-5α-cholane-21,22-dioate (*XXIV*)

Platinum oxide (50 mg) was added to a solution of unsaturated methyl ester *XVIII* (500 mg, 1·24 mmol) in ethyl acetate (30 ml) and hydrogenated for 2 h. The catalyst was filtered off and washed with ether. Crystallization of the residue after evaporation from light petroleum–ether gave 440 mg (88%) of saturated ester *XXIV*, m.p. 135—137°C, $[\alpha]_D^{25}$ −13° (*c* 1·47). IR spectrum (tetrachloromethane): 3 620, 3 470, 3 380 shoulder (OH), 1 761, 1 738 (—$CH(COOCH_3)_2$). ^{1}H-NMR spectrum: 3·68 s (6 H, 2 × $COOCH_3$), 3·55 m, (1 H, $C_{(3)}$—H), 3·32 d (1 H, $J = 11$, $C_{(20)}$—H), 0·78 s (3 H, $C_{(19)}$—H_3), 0·67 s (3 H, $C_{(18)}$—H_3). For $C_{24}H_{38}O_5$ (406·8) calculated: 70·90% C, 9·42% H; found: 70·60% C, 9·63% H.

Dimethyl 3β-Hydroxy-23,24-dinorchol-5-ene-21,22-dioate (*XVIII*)

A solution of butyllithium in hexane (1·87 ml, $c = 1{\cdot}6$ mol l^{-1}, 3 mmol) was added dropwise to a solution of diisopropylamine (303 mg, 3 mmol) in tetrahydrofuran (5 ml) under argon at −78°C. After 30 min stirring at −78°C further methyl ester *XXIII* (861 mg, 2 mmol, prepared according to ref.[12]) in tetrahydrofuran (4 ml) was added and the mixture was stirred for 40 min at −78°C. Methyl chloroformate (283·5 mg, 3 mmol) was added and stirring was continued for another 3 h at −78°C. The mixture was decomposed with a saturated ammonium chloride solution in water, the product was extracted with ether, the extract washed with ammonium chloride solution and evaporated. The residue (850 mg) contained according to TLC pure dimethyl ester *XVII*. ^{1}H-NMR spectrum: 5·32 m (1 H, $C_{(6)}$—H), 4·68 m (1 H, $C_{(2')}$—H from tetrahydropyranyl), 3·68 s (6 H, 2 × $COOCH_3$), 3·33 d (1 H, $J = 11$, $C_{(20)}$—H), 3·2—3·9 bm (3 H, $C_{(3)}$—H and $C_{(6')}$—H_2 from tetrahydropyranyl), 0·97 s (3 H, $C_{(19)}$—H_3), 0·80 s (3 H, $C_{(18)}$—H_3).

p-Toluenesulphonic acid monohydrate (250 mg) and water (1 ml) were added to a solution

of diester *XVII* (850 mg, 1·74 mmol) in a mixture of dichloromethane (20 ml) and methanol (20 ml) and the mixture was stirred for 2 h at room temperature. After dilution with ether (250 ml) it was washed with water, potassium hydrogen carbonate solution, water and evaporated. Crystallization of the residue from light petroleum–ether mixture hydroxy derivative *XVIII* was obtained (530 mg, 66%), with m.p. 152–154°C, $[\alpha]_D^{25}$ −68° (*c* 1·62). IR spectrum (tetrachloromethane): 3 620, 3 450 shoulder, 3 380 (OH); 3 025, 1 668 (C=C—H), 1 761, 1 738 (—CH(COOCH$_3$)$_2$). ^{1}H-NMR spectrum: 5·32 m (1 H, $C_{(6)}$—H), 3·66 s (6 H, 2 × $COOCH_3$), 3·50 m (1 H, $C_{(3)}$—H), 3·32 d (1 H, J = 11, $C_{(20)}$—H), 0·97 s (3 H, $C_{(19)}$—H_3), 0·70 s (3 H, $C_{(18)}$—H_3). For $C_{24}H_{36}O_5$ (404·6) calculated: 71·26% C, 8·97% H; found: 71·01% C, 8·86% H.

Diethyl 3β-Acetoxy-23,24-dinor-5α-chol-17(20)-ene-21,22-dioate (*XX*)

Palladium on charcoal (10%; 150 mg) was added to a solution of unsaturated ester *II* (500 mg, 1·06 mmol) in ethyl acetate (30 ml) and the mixture was hydrogenated for 4 h. The catalyst was filtered off and washed with ether. Crystallization of the residue after evaporation from ether gave 450 mg (90%) of ester *XX*, m.p. 142–144°C, $[\alpha]_D^{25}$ +42° (*c* 0·7). IR spectrum (tetrachloromethane): 1 732, 1 232 (CH_3COO—), 1 720 shoulder, 1 634 (unsaturated ester). ^{1}H NMR spectrum: 4·67 m (1 H, $C_{(3)}$—H), 4·25 q and 4·17 q (2 × 2 H, J = 7·2, 2 × —OCH_2CH_3), 2·85 m (2 H, $C_{(16)}$—H_2), 2·00 s (3 H, CH_3COO—), 1·32 t and 1·25 t (2 × 3 H, J = 7·2, 2 × —OCH_2CH_3), 0·96 s and 0·82 s (2 × 3 H, angular methyls). Mass spectrum (*m/z*): 474 (M^+). For $C_{28}H_{42}O_6$ (474·6) calculated: 70·86% C, 8·92% H; found: 70·73% C, 9·03% H.

Methyl 3β-Hydroxy-5α-pregnan-21-oate (*XXVII*)

A) Platinum oxide (20 mg) was added to a solution of methyl ester *XXII* (200 mg, 0·58 mmol) in ethyl acetate (20 ml) and the mixture was hydrogenated for 2 h. The catalyst was filtered off, washed with ether and evaporated. Crystallization of the residue from ether–light petroleum gave 160 mg (80%) of saturated ester *XXVII*. m.p. 126–128°C, $[\alpha]_D^{25}$ +3·5° (*c* 1·4). ^{1}H-NMR spectrum: 3·63 s (3 H, $COOCH_3$), 3·53 m (1 H, $C_{(3)}$—H), 2·23 m (2 H, $C_{(20)}$—H_2), 0·80 and 0·57 2 s (2 × 3 H, angular methyls). For $C_{22}H_{36}O_3$ (348·5) calculated: 75·82% C, 10·41% H; found: 76·00% C, 10·68% H.

B) Potassium hydroxide (500 mg) was added to a solution of unsaturated ester *II* (185 mg, 0·39 mmol) in ethylene glycol (11 ml) and the mixture was refluxed for 10 h. After cooling it was poured into water, acidified with hydrochloric acid and the product was extracted with ether. The extract was washed with water and evaporated, the residue was crystallized from methanol, to yield 100 mg (77%) of acid *IX*. IR spectrum (KBr): 3 408 (OH), 3 330–2 700, 1 685 (COOH), 1 648 (C=C). Sulfuric acid (100 mg) was added to a solution of unsaturated acid *IX* (90 mg, 0·27 mmol) in methanol (23 ml) and refluxed for 8 h. The mixture was evaporated under reduced pressure to 1/10 of its volume and partitioned between water (100 ml) and ether (50 ml). The ethereal layer was washed with a saturated potassium hydrogen carbonate solution and water and evaporated, the residue was chromatographed on silica gel (10 g) with benzene–ether (95 : 5). Yield, 75 mg (80%) of unsaturated methyl ester *X*. ^{1}H NMR spectrum: 5·56 t (1 H, J = 2·5, $C_{(20)}$—H), 5·37 m (1 H, $C_{(6)}$—H), 3·68 s ($COOCH_3$), 1·04 and 0·84 2 s (2 × 3 H, angular methyls). Platinum oxide (15 mg) was added to a solution of unsaturated methyl ester *X* (75 mg) which was hydrogenated for 2 h. The catalyst was filtered off and washed with ether. The residue of the filtrate after evaporation was crystallized from ether–light petroleum. Yield 40 mg (79%) of saturated ester *XXVII*, m.p. 125–128°C, which had identical properties as the product obtained under *A*.

17,20,21,22-Tetrahydroxy-3β-tetrahydropyranyloxy-17ξ-23,24-dinorchol-5-ene (*XXVIII*)

Diol *V* (552 mg, 1·28 mmol) was dissolved in a mixture of acetone (10 ml) and tetrahydrofuran (10 ml), and morpholine N-oxide (400 mg) and osmium tetroxide in 2-methyl-2-propanol (0·039 mol l^{-1}; 1 ml) were added to it. After stirring at room temperature for 30 min another portion of osmium tetroxide solution (0·5 ml) was added and the mixture stirred for another 3 h. Then sodium hydrogen sulfite (300 mg) in water (3 ml) was added and the mixture stirred for 30 min. Additional water (30 ml) was added and the mixture extracted with ethyl acetate (3 × 50 ml). The extract was dried and evaporated, yielding 309 mg (52%) of hydroxy derivative in the form of an amorphous powder, $[\alpha]_D^{25}$ −17° (*c* 0·35, pyridine, measured on a Perkin–Elmer 141 MC instrument). IR spectrum (KBr): 3 440 (OH), 1 138, 1 113, 1 059, 1 031, 978 (ether —O—). ^{1}H-NMR spectrum (Varian XL-200): 5·36 bm (1 H, $C_{(6)}$—H), 4·72 m (1 H, —O—CH—O from tetrahydropyranyl), 4·01, 3·71 and 3·94, 3·87 (4 H, $C_{(21)}$—H_2 + $C_{(22)}$—H_2, $J = 12$ and $J = 11$, respectively, calculated for an AB system), 3·50 bm (2 H, —O—CH_2— from tetrahydropyranyl), 2·37 m (2 H, $C_{(7)}$—H_2), 1·02 and 0·89 2 s (2 × 3 H, angular methyls). Mass spectrum (*m/z*): 362 (M − $C_5H_{10}O_2$), 271 (362 − $C_3H_7O_3$), 270 (362 − $C_3H_8O_3$), 85 (tetrahydropyrane, base peak). For $C_{27}H_{44}O_6$ (464·6) calculated: 69·80% C, 9·55% H; found: 69·52% C, 9·51% H.

21,22-Diacetoxy-17,20-dihydroxy-3β-tetrahydropyranyloxy-17ξ-23,24-dinorchol-5-ene (*XXIX*)

Hydroxy derivative *XXVIII* (230 mg, 0·5 mmol) was dissolved in a mixture of pyridine (10 ml) and acetic anhydride (0·5 ml). After 20 h standing at room temperature the mixture was partitioned between water (100 ml) and ether (40 ml) and the aqueous layers were extracted with three 20 ml portions of ether. The combined etheral extracts were washed with 5% hydrochloric acid and a saturated potassium hydrogen carbonate solution, dried and evaporated. The residue was chromatographed on two 20 × 20 cm silica gel thin-layer plates in benzene–ether (4 : 1). From the main zone 100 mg (35%) of diacetyl derivative *XXIX* were obtained in the form of an oil, $[\alpha]_D^{25}$ −44·1° (*c* 0·37). IR spectrum (tetrachloromethane): 3 595 (OH), 1 745, 1 232 (CH_3COO), 3 035 (=C—H), 1 138, 1 118, 1 059, 979 (—O—). ^{1}H-NMR spectrum (Varian XL-200): 5·35 m (1 H, $C_{(6)}$—H), 4·72 m (1 H, —O—CH—O— from tetrahydropyranyl), 4·44, 4·27 and 4·37, 4·28 (4 H, $C_{(21)}$—H_2 and $C_{(22)}$—H_2, $J = 12$ and $J = 11·5$ respectively, calculated for an AB system), 3·90 bm (1 H, $C_{(3)}$—H), 3·50 bm (2 H, —O—CH_2— from tetrahydropyranyl), 2·33 m (2 H, $C_{(7)}$—H_2), 2·11 and 2·105 2 s (2 × 3 H, CH_3COO), 0·99 and 0·92 2 s (2 × 3 H, angular methyls). Mass spectrum (*m/z*): 464 (M − C_5H_8O), 446 (464 − H_2O), 428 (446 − H_2O), 289 (446 − $C_7H_{11}O_3$), 271 (289 − H_2O), 253 (271 − H_2O). For $C_{31}H_{48}O_8$ (548·7) calculated: 67·86% C, 8·82% H; found: 68·18% C, 8·75% H.

23,24-Dinor-5α-cholane-3β,21,22-triol Triacetate (*XXVI*)

Lithium aluminum hydride (300 mg) was added to a solution of diester *XXIV* (406 mg, 1 mmol) in a mixture of tetrahydrofuran (30 ml) and 1,2-dimethoxyethane (10 ml) and refluxed under argon for 4 h. The mixture was decomposed with water and dilute hydrochloric acid, the product was extracted with ethyl acetate (5 × 100 ml) and the extract washed with water and evaporated. The residue was dissolved in pyridine (10 ml), acetic anhydride (5 ml) was added and the mixture allowed to stand at room temperature overnight. After pouring onto ice the separated product was filtered off under suction, washed with water, dried over phosphorus pentoxide and crystallized from methanol. Yield, 200 mg (42%) of triacetate *XXVI*, m.p. 98–101°C, $[\alpha]_D^{25}$ −8·5° (*c* 1·18). IR spectrum (tetrachloromethane): 1 739, 1 240 (CH_3COO). ^{1}H-NMR spectrum: 4·68 m (1 H, $C_{(3)}$—H), 4·10 m (4 H, $C_{(21)}$—H_2 and $C_{(22)}$—H_2), 2·03 s (9 H, 3 × CH_3COO),

0·80 s (3 H, $C_{(19)}$—H_3), 0·68 s (3 H, $C_{(18)}$—H_3). For $C_{28}H_{44}O_6$ (476·7) calculated: 70·56% C, 9·30% H; found: 70·31% C, 9·36% H.

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