SYNTHESES OF 17β-(2-MALEIMIDO)ANDROSTANES* **

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Starting from pregnane derivatives, 17β-(2-maleimido)androstanes of 5α- and Δ^5-series, with and without a 3β-acetoxy group, were prepared *via* methyl 3-androstanyl-3-cyano acrylates. The maleimidoandrostanes were synthesized as models of cardiotonic steroids.

In our earlier papers, we dealt with steroids compr sing a polysubstituted side chain[3-5] or a lactone ring in the 17 β-position[6]. In connection with this work and with a program[7,8] aimed at partial syntheses of cardenolides, we worked out a synthetic approach to derivatives of the types *IV*, *V* and *VI* characterized by the presence of a 17β-side chain with the same number of carbon atoms as in the lactone ring of cardenolides.

For the construction of the side chain we utilized a reaction applied by Kalvoda[9,10] in the syntheses of showdomycin and substituted maleimides. The reaction utilizes conversion of the acid chloride *II* into the nitril *III* which is without isolation condensed by Wittig reaction with methoxycarbonylmethylenetriphenylphosphorane to yield the substituted methyl cyanoacrylates *IV* and *V*. As starting materials served 3β-acetoxy-5-etienic acid[11,12] (*Ic*) prepared from 3β-acetoxy-5-pregnen-20-one and 3β-acetoxy-5α-etianic acid (*Ib*) prepared by acetylation of *Id* (ref.[14]) obtained from 3β-acetoxy-5α-pregnan-20-one[5,12]. The acid *Ia* (5α-etianic)[13] was prepared by alkaline hydrolysis of its methyl ester[5,15]. 3β-Hydroxy-5α-etianic acid[14] (*Id*) was also prepared in almost quantitative yield from methyl 3β-hydroxy-5-etienate[16-18] by its hydrogenation at atmospheric pressure in the presence of palladium on carbon in dioxane followed by hydrolysis of the saturated ester[14].

All three acids *Ia*, *Ib*, *Ic* were converted into the chlorides *II* by treatment with oxalyl chloride in benzene at room temperature. The product was subjected to substitution with hydrogen cyanide in the presence of excess methoxycarbonylmethylenetriphenylphosphorane which with the acyl cyanides *III* condenses to give a mixture of *E*- and *Z*-isomers of the corresponding methyl cyanoacrylates *IV* and *V*. As expected – and in accord with results obtained on model compounds[9-11] – in the

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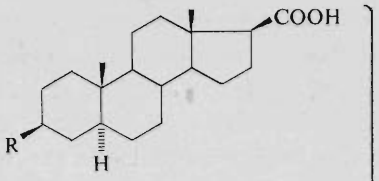
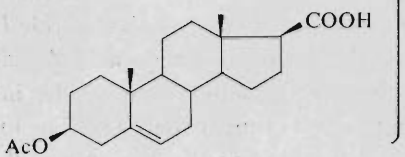
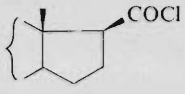
mixtures preponderate the *Z*-isomers *IV*, obviously for steric reasons. The ratio of *E*/*Z*-isomers was shown by thin-layer chromatography to be better than 1 : 10. The *E*- and *Z*-methyl cyanoacrylates can be separated by crystallization or chromatography on silica gel. The yields of the *Z*-isomers (capable of cyclization) ranged from 40 to 50%, based on the starting acid.



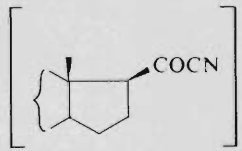
Ia, R = H
Ib, R = OAc
Id, R = OH



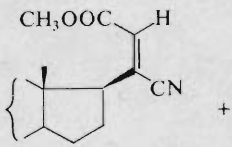
Ic



II

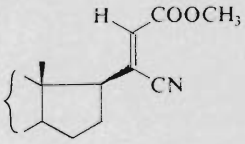


III

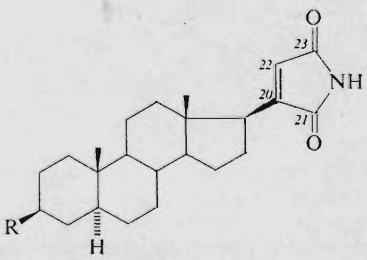


V

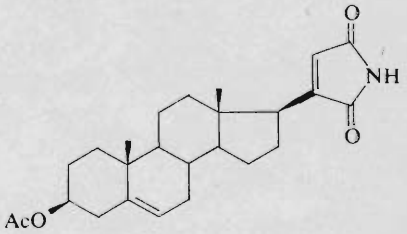
+



IV



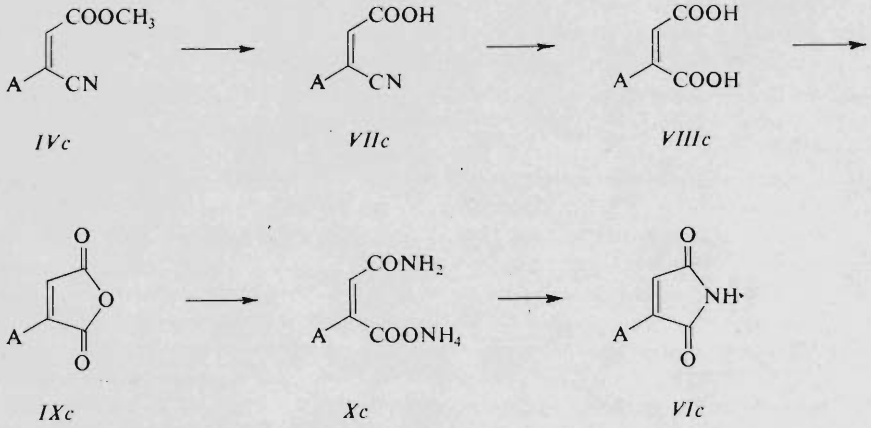
VIa, R = H
VIb, R = OAc
VId, R = OH



VIc

As shown by Kalvoda on his series of cyanoacrylates[9-11], derivatives of this type could be cyclized by treatment with a mixture of sulfuric acid, acetic acid and acetic anhydride (1 : 5 : 5) at elevated temperature (100°C, 20 min). In the case of the steroid derivatives, this method failed in the 3β-acetoxy-5-androstene series for a deep decomposition of the starting ester nitrile *IVc*. On the other hand, the procedure was applied without problems to the 5α-androstane series. It may be thus concluded that the decomposition of the afore-mentioned steroids is due to the presence of reactive groupings in the steroid skeleton and not to those in the side chain. This direct acid cyclization provided the cyclic imides *VIb* and *VIa* (in 19 and 71% yield, resp.). Since the work-up of the reaction mixture involves deacylation of the N-acyl derivative (formed as a side product) free hydroxy derivative *VId* is isolated in the 3β-substituted 5α-androstane series.

In the 3β-acetoxy-5-androstene series a different preparation of the imide[11] had to be chosen. Thus, the ester nitrile *IVc* was subjected to a two-step hydrolysis by treatment with aqueous sodium hydroxide followed by the action of aqueous acetic acid at elevated temperature to yield the diacid *VIIIc via* the nitrile acid *VIIc*. Dehydration of the diacid *VIIIc* by heating with acetic anhydride gave the cyclic anhydride *IXc*. Ammonolysis of the latter in benzene furnished the salt of the amido acid *Xc* which was treated with phosphorus pentoxide in dimethylformamide to give the imide *VIc* in 24·3% overall yield, based on the starting methyl cyanoacrylate *IVc*. The lower yield is obviously due to hydration of the 20,22 double bond in the course of the two-step hydrolysis.



A = 17β-(3β-acetoxy-5-androstenyl)-

In some respect, the imides reported in the present paper and cardenolides show a structural similarity; the carbon skeleton of the cyclic imide moiety in the former compounds is virtually identical with that of the lactone ring in the latter class of com-

pounds. They also show a notable structural similarity to analogous N-maleimido-androstane derivative that was synthesized[16] as a compound with potential biological activity for its ability to react with the SH group of biological systems. Methyl esters of substituted cyanoacrylic acids derived from steroids (*IV* and *V*) were also prepared as models for a synthesis of compounds with potential cardiotonic activity. This activity was demonstrated[17] for similar steroid derivatives of acrylic acid.

EXPERIMENTAL

Melting points were determined on a Boetius block. Optical rotations were measured on a polarometer Opton of the type VDRNA. The IR spectra were recorded on a Zeiss UR 20 spectrometer, ^{1}H-NMR spectra were recorded on a Tesla B 467 instrument (60 MHz) with tetramethylsilane as internal reference, chemical shifts are given in δ-scale, the coupling constants in Hz. The mass spectra were measured on an AEI MS 901 instrument. Analytical samples were dried at 50°C/ /25 Pa. The column chromatographies were carried out on silica gel according to Pitra, size of particles 60—120 μm (Service laboratories of the Institute). The thin-layer chromatography was carried out on silica gel G (Woelm) according to Stahl and the spots detected by spraying with sulfuric acid and heating. The solutions were concentrated or solvents removed on vacuum rotatory evaporator at 30—50°C/2·5 kPa.

(*Z*)-3β-Acetoxy-20-cyano-21-methoxycarbonyl-5α-pregn-20-ene (*IVb*)

A solution of methyl 3β-hydroxy-5-etienate[18] (1 g, 3 mmol) in dioxane (100 ml) was hydrogenated over palladium on carbon (10%; 0·5 g) catalyst with stirring at ambient temperature and atmospheric pressure for 20 h. The catalyst was filtered off, the solvent evaporated and the residue dried by addition of benzene and evaporation. Crystallization from methanol–ether gave almost quantitative yield of methyl 3β-hydroxy-5α-etianate, m.p. 174—175·5°C, identical with an authentic sample[5]. This material was dissolved in a mixture of benzene–methanol–water (100 ml), sodium hydroxide (250 mg) added and the mixture refluxed until no starting ester was present (40 h, checked by thin-layer chromatography). Acetic acid was added to the mixture until a pronounced acidic reaction was achieved, the solvent evaporated and the product dried in a desiccator over sodium hydroxide at 25 kPa. The residue (*c.* 1·5 g) containing the hydroxy acid *Id* was treated with pyridine (6·5 ml) and acetic anhydride (0·9 ml) and kept at room temperature with intermittent stirring for 18 h. After addition of water (1 ml), the mixture was refluxed for 5 min, more water (3 ml) added to the hot solution from which the acetylated acid *Ib* crystallized after standing. The latter was isolated, dissolved in benzene (15 ml), oxalyl chloride added (1 ml), and the mixture kept at room temperature for 2 h. The reaction course was monitored by thin-layer chromatography after dissolving a drop of the mixture in methanol; when no more *Ib* was pressent, the volatile components of the mixture were removed at reduced pressure, dichloromethane was added and evaporated three times. The residue was dissolved in dichloromethane (6 ml), the solution was placed in an ice bath and a solution of methoxycarbonylmethylenetriphenylphosphorane (2·22 g, 6·64 mmol) and liquid hydrogen cyanide (0·56 ml, dried with calcium chloride) in dichloromethane (15 ml) was added in several portions. After 2 h at room temperature the mixture was evaporated and the residue chromatographed on a column of silica gel in light petroleum–ethyl acetate 9 : 1 to yield the ester nitrile *IVb* (465 mg, 39·5%), m.p. 189·5—192·5°C. $[\alpha]_D^{25}$ +10° (*c* 1·8; chloroform), IR spectrum (chloroform): 2230 cm^{-1} (CN), 1 725, 1 259, 1 030 cm^{-1} (CH_3COO), 1 715, 1 627, 1 440 cm^{-1} ($COOCH_3$),

^{1}H-NMR spectrum (deuteriochloroform): 6·38 s (1 H, $C_{(22)}$—H), 4·72 m (1 H, $C_{(3)}$—H), 3·84 s (3 H, CH_3O), 2·03 s (3 H, $OCOCH_3$), 0·87 s (3 H, CH_3), 0·67 s (3 H, CH_3). For $C_{26}H_{37}NO_4$ (427·6) calculated: 73·04% C, 8·72% H, 3·28% N; found: 73·19% C, 8·85% H, 3·28% N.

(20*Z*)-3β-Acetoxy-20-cyano-21-methoxycarbonyl-pregna-5,20-diene (*IVc*)

A solution of the acid *Ic* (ref.[12], 3·6 g, 10 mmol) in benzene (50 ml) was treated with oxalyl chloride (2·7 ml, 300%) while stirring for 2 h at room temperature. (For checking the reaction course by thin-layer chromatography a drop of the reaction mixture was treated with methanol.) After this time, no starting material was present, the volatile components were removed at reduced pressure, the residue was dissolved in dichloromethane, the latter evaporated three times, the residue was dissolved in dichloromethane (20 ml) and cooled to 0°C. To this solution was added an ice-cooled mixture of methoxycarbonylmethylentriphenylphosphorane (8 g, 22·5 mmol) and liguid hydrogen cyanide (2 ml, dried with calcium chloride) in dichloromethane (50 ml) and kept at room temperature for 1 h. The reaction mixture was evaporated and co-evaporated with dichloromethane three times and the product chromatographed on a silica gel column (250 g) in light petroleum–ethyl acetate 9 : 1 to give the ester nitrile *IVc* which was crystallized from benzene–methanol to furnish pure *IVc* (2·2 g, 48·2%), m.p. 191—193°C, $[\alpha]_D^{25}$ —77° (*c* 0·88; chloroform). IR spectrum (chloroform): 2230 cm^{-1} (CN), 1728 cm^{-1} (CO of CH_3COO and $COOCH_3$), 1255, 1028 cm^{-1} (CH_3COO), 1439 cm^{-1} ($COOCH_3$), 1626 cm^{-1} (C=C). ^{1}H-NMR spectrum (deuteriochloroform): 6·35 s (1 H, $C_{(22)}$—H), 5·49 (1 H, $C_{(6)}$—H), 4·59 m (1 H, $C_{(3)}$—H), 3·82 s (3 H, CH_3O), 2·02 s (3 H, CH_3COO), 1·07 s (3 H, CH_3), 0·77 s (3 H, CH_3). For $C_{26}H_{35}NO_4$ (425·6) calculated: 73·38% C, 8·29% H, 3·29% N; found: 73·11% C, 8·25% H, 3·47% N.

(*Z*)-20-Cyano-21-methoxycarbonyl-5α-pregn-20-ene (*IVa*)

Methyl-5α-etianate[5] (4·22 g, 13·3 mmol) was dissolved in a mixture of ethanol (15 ml), benzene (5 ml) and water (2 ml), sodium hydroxide (1·25 g) added and the mixture stirred and refluxed for 40 h, neutralized with acetic acid and evaporated. The residue was dissolved in a mixture of acetone and ethanol and precipitated with water. The precipitate was filtered, washed with water and dried. The acid *Ia* showed a m.p. (233—235°C) slightly higher than reported in the literature[13]. The free acid *Ia* was dispersed in benzene (70 ml) and after addition of oxalyl chloride (4·1 ml) left standing until the thin-layer chromatography indicated completion of the reaction. (Before application, a sample was treated with methanol.) After removal of the volatile components at reduced pressure, the residue was evaporated with benzene twice, dissolved in dichloromethane (20 ml), the solution cooled in an ice bath and a solution of methoxycarbonylmethylenetriphenylphosphorane (12 g, 36 mmol) and liquid hydrogen cyanide (3 ml, dried with calcium chloride) in dichloromethane (50 ml) was added in several portions. The mixture was kept at 0°C for 30 min, then at room temperature for 2 h, the volatile components removed at reduced pressure, the residue evaporated with dichloromethane twice, roughly purified on a small silica gel column (50 g) in light petroleum–ether (4 : 1) and rechromatographed on silica gel (200 g) column using the same solvent system. The ester nitrile *IVa* thus obtained was crystallized from ether–light petroleum to give pure *IVa* (2·8 g, 50·5%), m.p. 184—185°C, $[\alpha]_D^{25}$ +1·7° (*c* 2·4; chloroform). IR spectrum (chloroform): 1730, 1440, 1245, 1151 cm^{-1} ($COOCH_3$), 1628 cm^{-1} (C=C), 2230 cm^{-1} (CN). ^{1}H-NMR spectrum (deuteriochloroform): 6·32 s (1 H, $C_{(22)}$—H), 3·81 s (3 H, CH_3O), 0·78 s (3 H, CH_3), 0·71 s (3 H, CH_3). Mass spectrum (*m/z*): 369 (M^+), 354 (M—CH_3), 217 ($C_{16}H_{25}$). For $C_{24}H_{35}NO_2$ (369·5) calculated: 78·00% C, 9·55% H, 3·79% N; found: 78·28% C, 9·58% H, 3·70% N.

17β-(2-Maleimido)-5α-androstane (*VIa*)

A mixture of ester nitrile *IVa* (600 mg, 1·62 mmol), glacial acetic acid (5 ml), acetic anhydride (5 ml) and concentrated sulfuric acid (1 ml) was heated at 100°C for 20 min, poured into water (150 ml) containing pyridine (5 ml) and the product taken up in ethyl acetate (three 50 ml portions). The joint extracts were washed with hydrochloric acid (5%) and sodium hydrogen carbonate, evaporated and the residue dissolved in a mixture of benzene (15 ml) and methanol (20 ml) and treated overnight with hydrochloric acid (37%, 0·7 ml) at room temperature. Evaporation and crystallization of the residue from acetone–benzene gave the imide *VIa* (410 mg, 71%), m.p. 276–279°C, $[\alpha]_D^{25}$ −56° (*c* 1·2; pyridine). IR spectrum (chloroform): 1 778, 1 729 cm^{-1} (C=O), 1 623 cm^{-1} (C=C), 3 448 cm^{-1} (NH). ^{1}H-NMR spectrum (tetrachloromethane and hexadeuteriodimethyl sulfoxide); 6·29 s (1 H, $C_{(22)}$—H), 0·75 s (3 H, CH_3), 0·60 s (3 H, CH_3). Mass spectrum (*m/z*): 355 (M^+), 340 (M−CH_3), 245 ($C_{18}H_{29}$), 217 ($C_{16}H_{25}$). For $C_{23}H_{33}NO_2$ (355·5) calculated: 77·70% C, 9·36% H, 3·94% N; found: 77·74% C, 9·35% H, 3·82% N.

3β-Hydroxy-17β-(2-maleimido)-5α-androstane (*VId*)

A mixture of the ester nitrile *IVb* (300 mg, 0·7 mmol), glacial acetic acid (2·5 ml), acetic anhydride (2·5 ml) and concentrated sulfuric acid (0·5 ml) was heated at 100°C for 30 min, poured into water and the product extracted with ethyl acetate, the joint extracts washed with sodium hydrogen carbonate solution, dried with sodium sulfate and the solvent evaporated. The residue was coevaporated with xylene, dissolved in methanol (10 ml), hydrochloric acid (37%, 0·3 ml) was added and the solution was left overnight at room temperature. After evaporation, the residue was chromatographed on a loose layer of silica gel (50 g) using benzene–ethyl acetate (8 : 2) for development. The band of R_F *c.* 0·2 was eluted with chloroform and crystallized from the same solvent to give the imide *VId* (50 mg, 19%), m.p. 135–138°C, $[\alpha]_D^{25}$ +27° (*c* 0·88; pyridine). IR spectrum (chloroform): 3 410 cm^{-1} (NH), 1 773, 1 723, 1 712 cm^{-1} (imide), 1 638 cm^{-1} (C=C), 3 615, 1 048 cm^{-1} (OH). ^{1}H-NMR spectrum (deuteriochloroform): 5·87 br. s (1 H, $C_{(22)}$—H), 3·39 (1 H, $C_{(3)}$—H), 0·80 s (3 H, CH_3), 0·66 s (3 H, CH_3). Mass spectrum (*m/z*): 371 (M^+), 353 (M−H_2O), 338 (M−H_2O−CH_3). For $C_{23}H_{33}NO_3$ (371·5) calculated: 74·36% C, 8·95% H, 3·77% N; found: 74·64% C, 8·65% H, 3·58% N.

3β-Acetoxy-17β-(2-maleimido)-5-androstane (*VIc*)

Water (20 ml) and 1M-NaOH (1·5 ml) was added to a solution of *IVc* (435 mg, 1 mmol) in tetrahydrofuran (50 ml). The mixture was heated at 100°C for 8 h, acidified with excess of acetic acid and heated again at 100°C for 10 h, the solvents removed under reduced pressure and dried at a vacuum of 25 Pa. After addition of acetic anhydride (10 ml), the residue was heated to 100°C for 1 h, the solvent removed and the residue dried azeotropically with xylene, dissolved in benzene and saturated with gaseous ammonia (an aqueous extract of a sample showed a strongly basic reaction). The saturated solution was left overnight and evaporated. The residue was treated with a mixture of phosphorus pentoxide (1·5 g) in dimethylformamide (5 ml) at 90°C for 4 h, poured on ice and the product taken up in chloroform, the extract dried with magnesium sulfate and the solvent removed *in vacuo*. The residue was chromatographed on a loose layer of silica gel (50 g), using benzene–ethyl acetate (9 : 1) for development, and yielded the imide *VIc*. Crystallization from light petroleum–acetone–ether gave pure *VIc* (100 mg, 24·3%), m.p. 261–263·5°C, $[\alpha]_D^{25}$ −115° (*c* 1·0; chloroform). IR spectrum (chloroform): 3 445, 1 775, 1 728 cm^{-1} (imide), 1 728, 1 255, 1 034 cm^{-1} ($OCOCH_3$), 1 621 cm^{-1} (C=C). ^{1}H-NMR spectrum (deuteriochloroform): 7·58 broad (1 H, NH), 6·30 s (1 H, $C_{(22)}$—H), 5·39 m (1 H, $C_{(6)}$—H), 4·58 m (1 H,

$C_{(3)}$—H), 2·02 s (3 H, $OCOCH_3$), 1·01 s (3 H, CH_3), 0·62 s (3 H, CH_3). Mass spectrum (*m/z*), 351 ($(M-60)^+$ $C_{23}H_{29}NO_2$), 213 ($C_{16}H_{21}$). For $C_{25}H_{33}NO_4$ (411·5) calculated: 72·96% C, 8·08% H, 3·40% N; found: 72·75% C, 7·93% H, 3·56% N.

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