

## CARDENAMIDES FROM CARDENOLIDES: CARDIAC AND ANTICANCER ACTIVITIES<sup>+</sup>

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Two ways are shown to transform cardioactive cardenolides into cardenamides [17 $\beta$ -(5-oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ ,14 $\beta$ -androstane derivatives], and their derivatives by replacement of their ring oxygen by N-R. Cardioactivity is strongly decreased by this transformation. The comparatively easily accessible 21-oxocardenamides [17 $\beta$ -(2-maleimidyl)-steroids] are strongly thiol reactive and show remarkable anticancer activity.

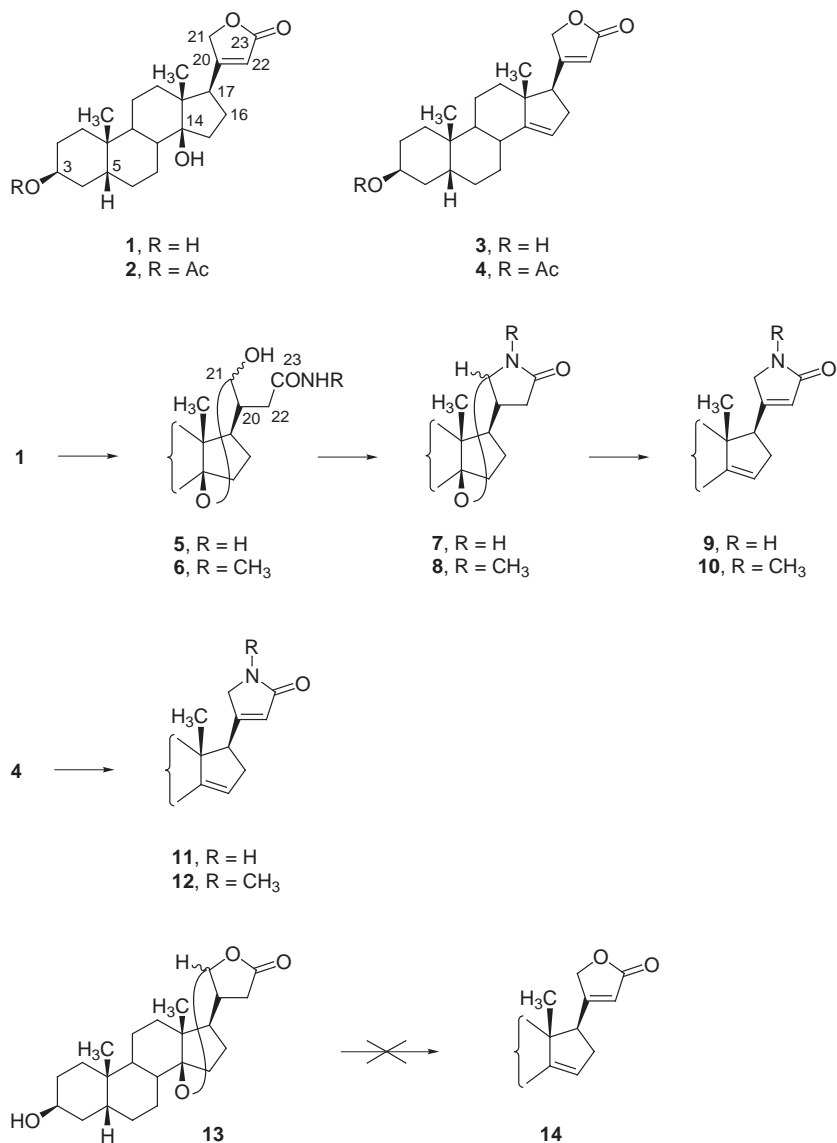
**Keywords:** Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibition; Cardenolides; Cardenamides; Steroids; Lactams; 21-Oxocardenamides; Anticancer activity.

Cardenolides play a central role in the treatment of cardiac failure until today. Their main drawback is their narrow therapeutic range making an optimal treatment very difficult. This stimulates a search for better drugs for replacing of cardenolides – but so far without a breakthrough<sup>1</sup>. Therefore, we have tried to replace the ring oxygen of the cardenolide lactone ring by N-R to get analogous lactams (cardenamides, 17 $\beta$ -(5-oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ -androstane derivatives) and their derivatives and to see their influence on the cardioactivity as measured by the inhibition of guinea pig and/or human cardiac ATPase.

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### First Method

The cardenolide digitoxigenin **1** (Scheme 1) reacts at room temperature with ammonia or methylamine in methanol to the hemiacetal amide **5** (yield 53%) or **6** with lactone ring opening, shift of the C20–C22 double



SCHEME 1

bond under formation of an aldehyde group at C21 and hemiacetal formation with the 14-OH group (isomerization), which subsequently condenses to the 14,21-epoxylactam **7** or **8** (refs<sup>2,3</sup>). With aniline, the lactone ring is not attacked even at 100 °C.

From compounds **5–8** the cardenamides **9** or **10** can be formed by treatment with  $\text{SbCl}_3/\text{SiO}_2$  at 120 °C (54%) or ethanol/ $\text{H}_2\text{SO}_4$  (0.2%) under reflux<sup>4</sup> (33%). Unfortunately, these procedures are also associated with the loss of 14 $\beta$ -OH and formation of the  $\Delta^{14}$ -double bond which is unfavourable for cardioactivity (*cf.* Table I: **1** vs **3**; **2** vs **4**).

TABLE I

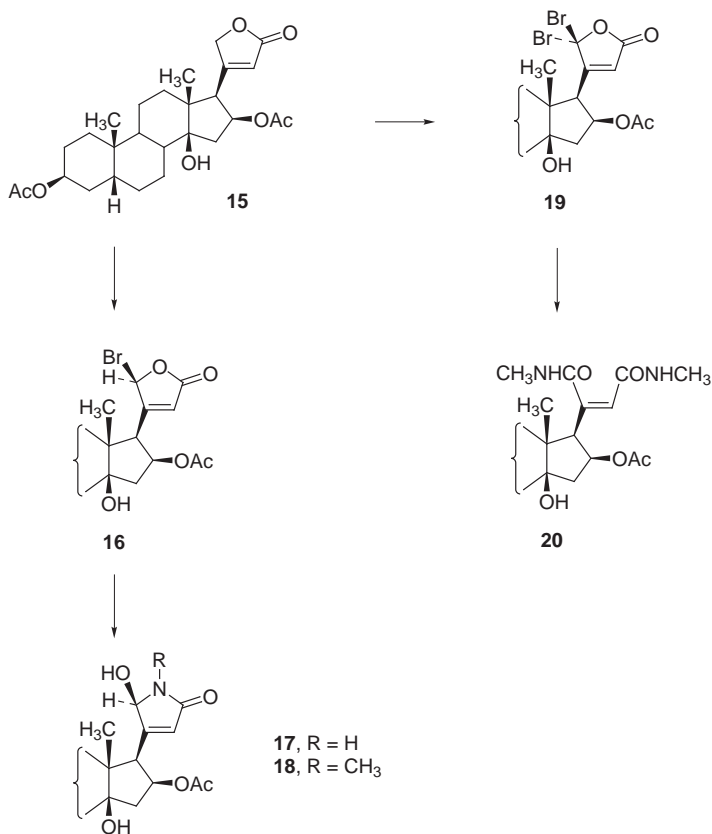
Inhibition of the  $\text{Na}^+,\text{K}^+$ -ATPase of guinea pig heart (GPH) or human heart (HH) by lactones and lactams (n.d. means not determined)

Compound	GPH		HH
	concentration, $\mu\text{mol l}^{-1}$	inhibition, %	$I_{50}$ , $\mu\text{mol l}^{-1}$
<b>1</b>	1.39	50	0.053
<b>2</b>	$1.23 \pm 0.14$	50	0.041
<b>3</b>	10	15	n.d.
<b>4</b>	100	13	n.d.
<b>9</b>	50	7	n.d.
<b>10</b>	100	~35	n.d.
<b>15</b>	$1.03 \pm 0.19$	50	0.19
<b>17</b>	100	35	n.d.
<b>18</b>	100	~30 $\pm$ 1	65.8
<b>20</b>	100	28	n.d.
<b>22</b>	~75 $\pm$ 5	50	n.d.
<b>23a</b>	100	28 $\pm$ 4	n.d.
<b>23</b>	~56 $\pm$ 4	50	n.d.
<b>24</b>	100	30 $\pm$ 1	n.d.
<b>25</b>	100	15	n.d.
<b>26</b>	76	50	65.8
<b>27</b>	~100	50	66
<b>28</b>	27	50	n.d.
<b>30</b>	n.d.	n.d.	45

These transformations are restricted to the lactam derivatives: The oxygen analogue isodigitoxigenin **13** is not transformed into the  $\Delta^{14}$ -butenolide **14** under these conditions.  $14\beta$ -OH is not a prerequisite for the formation of the  $\Delta^{14}$ -cardenamides. Their formation (**11**, **12**) works as well starting with  $\Delta^{14}$ -cardenolides (**3**, **4**) under the same conditions.

### Second Method

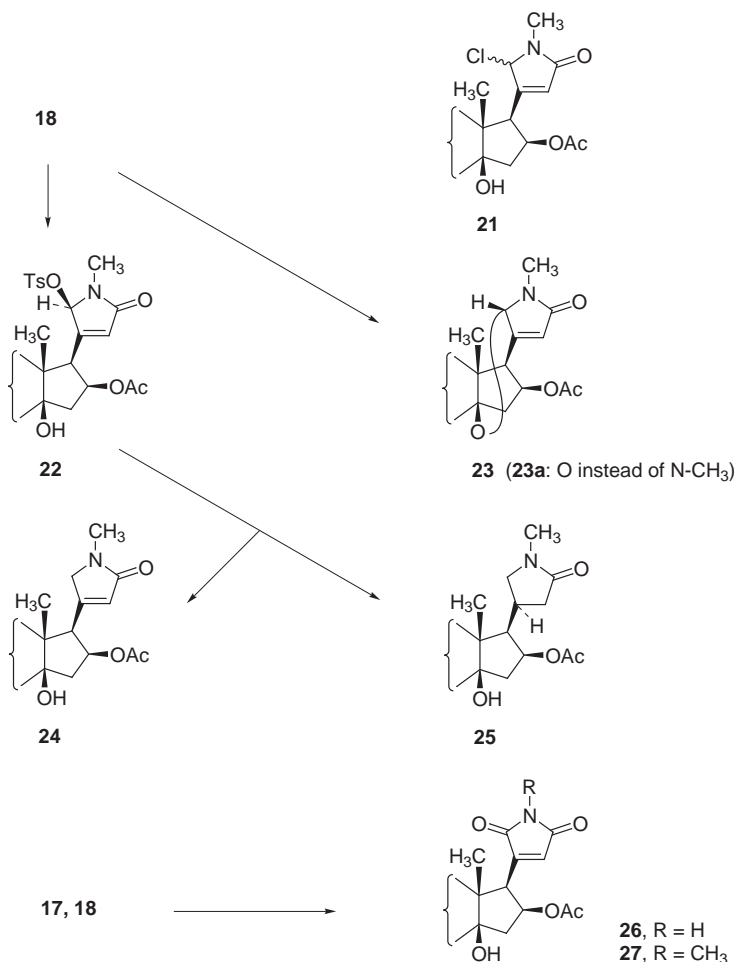
Avoidance of isomerization and conservation of  $14\beta$ -OH can be achieved by reaction of the  $(21R)$ -21-bromocardenolide **16** (Scheme 2) (from **15** by bromination with *N*-bromosuccinimide<sup>5</sup>) with ammonia or methylamine leading to the  $(21R)$ -21-hydroxycardenamide **17** (51%) or the  $(21R)$ -21-



SCHEME 2

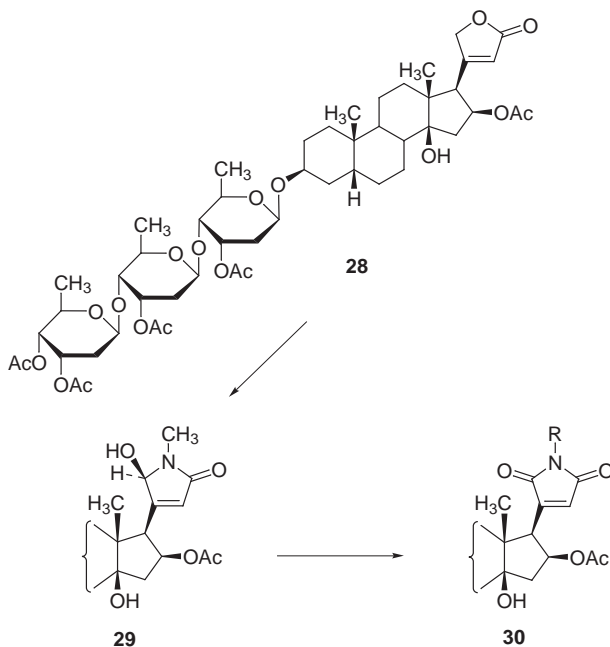
hydroxy-*N*-methylcardenamide **18** (60%) containing the C20–C22 double bond and 14 $\beta$ -OH but an additional OH group at C21 (ref.<sup>6</sup>).

Treatment of **17** or **18** with NaBH<sub>4</sub> does not remove the 21-OH group. Treatment of **18** with SOCl<sub>2</sub> does not lead to the formation of the respective 21-Cl derivative **21** (Scheme 3) but of the (21*S*)-14,21-epoxylactam **23**. To remove 21-OH from **18** it is reacted with tosyl chloride to the (21*R*)-tosylate **22** (48%) followed by reduction with zinc/acetic acid to give the 14 $\beta$ -hydroxy-*N*-methylcardenamide<sup>6</sup> **24** (43%) and its 20(22)-dihydro derivative (**25**; 17%).



SCHEME 3

Otherwise, oxidation<sup>7</sup> of the allylic 21-OH of **17** or **18** with active  $\text{MnO}_2$  or  $\text{CrO}_3 \cdot 2\text{Py}$  leads to the 21-oxo derivative **26** (72%) or **27** (70%). In an analogous way **30** (31% from **28**) is prepared from pentaacetylgitoxin **28** in order to elucidate the role of the sugar chain<sup>8</sup> (Scheme 4). The 21,21-dibromogitoxigenin diacetate<sup>9</sup> **19** (Scheme 2) reacts with methylamine to the ring-opened 21,23-bis-(*N*-methylamide)<sup>10</sup> **20**.



SCHEME 4

### Properties of the Compounds

The following properties of the compounds are in agreement with the given structures. The chromatographic mobility increases in the following order: diamide **20** < 21-OH-lactams **17** < **18** < *N*-Me-lactam **24** < maleimide **26** < lactone **15** < maleimide **27** < 21-tosylate **22** < 14,21-*O*-*N*-Me-lactam **23** < 14,21-*O*-lactone **23a**. This means that lactams and maleimides are more polar than analogous lactones. Thiol reactivity: **26**, **27**, **30** are maleimide derivatives and as such add, SH groups to the C20–C22 double bond as shown by treatment with  $\text{H}_2\text{S}$ /pyridine at 0 °C: **26** and (somewhat faster) **27** form a more polar, sulfur-containing product (TLC). Colour reactions: Like the cardenolide ring, the cardenamide ring, too, reacts with 1,3-dinitrobenzene/NaOH (Raymond reaction<sup>11</sup>) with the formation of a coloured

(violet and red, respectively) Meisenheimer complex, making a specific detection in TLC easy **9**, **10**, **11**, **12**, **24**, as far as there are two hydrogens bound at C21. Cardenamides with an NH group are detected by the chlorine-benzidine reaction<sup>12</sup>. Lactone transformation into lactams shifts the wavelengths of the UV maxima by about 10 nm to shorter wavelengths, which is compensated or overcompensated by 21-substituents. Wavelengths of the UV maxima increase in the following order: lactams **9**, **24** < 21-OH-lactams **17**, **18** < lactones **1**, **2**, **15**, **23a** < diamide **20** < 21-tosylate **22** < maleimide **27**. The molar absorption coefficients are nearly equal for all the compounds. In the IR spectra ( $\nu$  values given in  $\text{cm}^{-1}$ ) the transition from lactone to lactam (**15**  $\rightarrow$  **24**) shifts the O-H-frequency to lower values, the C=O-frequency is not strongly affected except for the lowering in the case of the maleimides **26** and **27**. An amide band is found between 1 670 and 1 700 which is missing in case of the maleimides **26** and **27**. The C=C frequency between 1 600–1 635 is strongly shifted to 1 690 by 14,21-epoxide formation in case of **23**. In MS spectra most compounds (**9**, **20**, **23**, **24**, **26**) show the molecular ion, others split off  $\text{H}_2\text{O}$  or  $\text{AcOH}$  (**17**, **18** or **27**). The lactam ring is especially stable as is seen in MS of **9**: here the molecular peak is the basic peak. In  $^1\text{H}$  NMR spectra only lactam ring protons are shifted compared with lactones. Comparing **15** and **24** the values for C21- $\text{H}_2$  are lowered for about 1 ppm. In the presence of a C14–C15 double bond the same holds true (**3** vs **9**; **4** vs **11**; **4** vs **12**). These shifts are more or less compensated by substituents at C21 (**17**, **18**, **20**, **23**).

### *Biological Activity*

#### $\text{Na}^+/\text{K}^+$ -ATPase Inhibition and Guinea Pig Auricle Test

As a measure of cardioactivity the inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase ( $\text{Na}^+/\text{K}^+$ -transporting adenosine triphosphatase, E.C.3.6.1.37) of guinea pig heart (GPH) and/or human heart<sup>13,14</sup> (HH) were determined. The obtained values (Table I) are limited in number and, therefore, allow only preliminary conclusions as to more general structure-activity relationships. The change from lactone to lactam decreases the activity of the very active **15** by more than two orders of 10 (**15** vs **24**), also in the human heart (**15** vs **26**, **27**, **30**), but moderate for the least active **28** (**28** vs **30**). The activity of the little active **3** is nearly unchanged (**3** vs **9**, **10**), but increased for the low active **23a** (**23a** vs **23**, the most active lactam shown here). The change of NH by N-methyl has no influence (**9** vs **10**; **17** vs **18**) or decreases the activity

slightly (**26** vs **27**). The hydrogenation of the C20–C22 double bond in the lactam ring decreases the activity (**24** vs **25**) as does the opening of the lactame ring (**27** vs **20**). Contrary to the experience in the cardenolide field, substitution at C21 has no influence (**24** vs **18**) or increases the activity (**23a** vs **23**; **24** vs **22**; **24** vs **27**). The sensitivity of human heart Na<sup>+</sup>/K<sup>+</sup>-ATPase is somewhat higher than that of guinea pig heart (**27**). Also in the isolated guinea pig auricle<sup>15</sup>, **18** shows a very weak activity<sup>16</sup> (Table II).

### Anticancer Activity

The anticancer activity of maleimide derivatives **26**, **27**, **30** was tested in three cancer cell lines: Ehrlich mouse ascites tumor cells (EMAC), mouse melanosarcoma cells (B16) and human mammary carcinoma cells<sup>8</sup> (MCA). The results are shown in Table III. For EMAC cells **30** is the most active in-

TABLE II

Inotropic and arrhythmogenic activity of the 21-hydroxy-*N*-methylactam **18** on the isolated guinea pig auricle<sup>16</sup> (method<sup>15</sup>)

Concentration, $\mu\text{mol l}^{-1}$	$I_t$ , % <sup>a</sup>	$A_t$ , % <sup>b</sup>
<b>3</b>	3.8	0
<b>6</b>	6.6	11
<b>12</b>	11.1	6.3

<sup>a</sup>  $I_t$  means increase in the inotropic effect; <sup>b</sup>  $A_t$  means frequency of arrhythmia.

TABLE III

Anticancer activity (n.d. means not determined)

Compound	Concentration $\mu\text{mol l}^{-1}$	Cell proliferation inhibition, %			Na <sup>+</sup> /K <sup>+</sup> -ATPase inhibition $H_{50}$ , $\mu\text{mol l}^{-1}$	
		EMAC	B16	MCA	EMAC	NCT
<b>26</b>	12	50	n.d.	n.d.	n.d.	76 <sup>a</sup>
<b>27</b>	6.7	50	12.6 ± 2.2	56.7 ± 9.8	>>30 <sup>b</sup>	66 <sup>c</sup>
<b>30</b>	1.5	50	1.5	n.d.	89	45 <sup>a</sup>

<sup>a</sup> Guinea pig heart; <sup>b</sup> low solubility did not allow a more precise determination; <sup>c</sup> human heart.



hibitor, demonstrating the favourable effect of the acetylated sugar chain, contrary to its effect in  $\text{Na}^+/\text{K}^+$ -ATPase inhibition. Comparison of **26** and **27** reveals the favourable effect of *N*-methyl in comparison with NH. Compound **27** is less active against B16 but more and equally active against EMAC and MCA. The anticancer active concentrations are far below cardioactive concentrations (*cf.*  $\text{Na}^+/\text{K}^+$ -ATPase inhibition). It seems that the anticancer activity is caused by the thiol reactivity of the compounds. The  $\text{Na}^+/\text{K}^+$ -ATPase inhibition by **30** is in GPH twice as strong as in EMAC.

## Discussion

Shortly after the synthesis of the cardenamides<sup>4,6</sup> **9–12**, **24** shown here, two other syntheses<sup>17,18</sup> were published independently. These methods have the same or a higher number of steps and give about the same yield of cardenamides showing a strongly decreased cardiac activity<sup>18</sup>.

The reaction mechanisms of the first and second method (this paper) have the first step in common: the aminolysis of the lactone ring leading to the 23-amide<sup>2,3</sup>, but they differ strongly in the reaction rate with amines, depending on the substitution at C21. The reaction times are:

for 21- $\text{H}_2$ : >21days, for 21-Br,H:  $\leq 2$  h, for 21- $\text{Br}_2$ :  $\leq 1$  min (!)

as measured by consumption of the starting material, thus demonstrating a steep increase in the reactivity caused by the *-I*-effect of 21-Br, which increases the positive partial charge at C23.

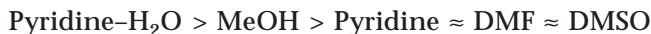
Further influences are the cardenolide structure,



the basicity of the amine



and the solvent used



showing clearly that protic solvents are favourable.

Both methods, however, differ markedly in the following steps:

In the first method, the primarily formed 23-amide undergoes isomerization to **5**, **6**. Only here the lactam (cardanamide) formation<sup>2,3</sup> (**7**, **8**)

seems possible. Dehydration of these compounds under acid or Lewis acid catalysis leads to the cardenamides<sup>4</sup> **9–12**.

The easy hydrolysis of cardanolides may be the cause that the oxygen analogue **13** does not form the respective lactone **14** under these conditions.

In the second method isomerization is possible in principle, but is overcome by the much faster S<sub>N</sub>2 reaction of the C23-amide group with the bromo substituent at C21 with Walden inversion forming only the 21*R* epimers of the 21-hydroxycardenamides<sup>6,8</sup> (**17**, **18**) (only one spot found in TLC). The 21*R* configuration is deduced from the easy formation of the (21*S*)-14,21-epoxylactam **23** in analogy to the behaviour of the (21*R/S*)-21-bromocardenolides: Only the (21*R*)-21-bromo derivative **16** forms quite easily the (21*S*)-14,21-epoxide **23a**. The 17β-configuration (excluding epimerization at C17 under amine treatment) of the cardenamide ring is concluded from <sup>1</sup>H NMR (17α-H) and again the easy formation of **23**. In 17α-cardenolides the 14,21-epoxide formation is impossible for steric reasons.

The reaction of the 21,21-dibromocardenolide<sup>9</sup> **19** (Scheme 2) with methylamine leads to the respective 23-*N*-methylamide which forms the 21-COBr derivative by loss of HBr. This does not react with the 23-*N*-methylamide group by ring closure with formation of **27** (as can be deduced from different TLC mobility), but with the stronger nucleophile methylamine under formation of the 21,23-diamide<sup>10</sup> **20**. The amide bands I and II in the IR spectrum of **13** are incompatible with the structure of a 21,21-bis(methylamino)cardenolide (*cf.* Experimental).

### Removal of 21-OH

In 21-hydroxycardenolides, 21-OH is easily removed by treatment<sup>19</sup> with NaBH<sub>4</sub>. This fails with 21-hydroxycardenamides because of their less easy hydrolysis which might be a prerequisite for the removal. Therefore, the reduction of the 21-tosylate was used which is accompanied in part by hydrogenation of the C20–C22 double bond, leading to the 20(22)-dihydro derivative of **24** (**25**).

### Cardioactivity

The reason for the drop in activity of cardenamides compared to cardenolides (Table I) is not clear at present but it demonstrates the importance of the ring oxygen in the lactone ring for the activity. Because NH and

*N*-methyl derivatives do not differ essentially in their activity (Table I) additional hydrogen bond formation by NH is not responsible.

X-Ray structures of **15** (ref.<sup>20</sup>) and **26** (ref.<sup>21</sup>) do not show serious differences with respect to bond lengths and angles. A possible reason might be the change in the D-ring shape (in **15**: 13 $\alpha$ ,14 $\beta$ -half chair, in **26**: between 13 $\alpha$ ,14 $\beta$ -half chair and 14 $\beta$ -envelope), as long as it also exists in solution. 17 $\alpha$ -Configuration as a possible reason for loss of activity is likewise excluded as shown above (17 $\alpha$ -cardenolides are known as inactive). It is remarkable that, contrary to the cardenolide series, the presence or absence of 14 $\beta$ -OH has no influence on the activity. Therefore, an electronic effect in the cardenamide ring could be taken into consideration. Changes in the wavelengths of the UV-absorption maximum reflect electronic changes in this chromophore. In this connection it is interesting to see the parallelism between the wavelength of UV-absorption maxima and biological activity: lactams ( $\approx$ 205 nm), low activity; lactones ( $\approx$ 217 nm), active; bufadienolides ( $\approx$ 300 nm), more active.

### Anticancer Activity

The thiol reactivity of the maleimide derivatives **26**, **27**, **30** is similar to the well-known maleimide derivative showdomycin<sup>22</sup>. In this compound, the maleimide ring is linked to a ribofuranosyl residue in a "C-glycosidic" manner. This compound is well known as a broad-spectrum antibiotic and shows furthermore other biologic activities including antitumor activity<sup>22</sup>. Compounds **26**, **27**, **30** also show this activity which seems to be attractive for an in-depth investigation. In comparison to showdomycin, **30** is much less polar which causes a strongly different distribution in the body. The question of additional activities needs further research.

### EXPERIMENTAL

Thin layer chromatography (TLC) was done on TLC plates (Kieselgel 60 F254 Merck). Mobile phases: a, chloroform-acetone (95 : 5); b, chloroform-acetone (90 : 10); c, chloroform-acetone (70 : 30); d, chloroform-ethanol (97.5 : 2.5); e, chloroform-ethanol (95 : 5); f, chloroform-ethanol (80 : 20). In brackets (*n* ×): number of developments if *n* > 1. Detection: H<sub>3</sub>PO<sub>4</sub>/UV 266 nm (ref.<sup>23</sup>), 1,3-dinitrobenzene (*m*-DNB)/NaOH (ref.<sup>11</sup>), fluorescein/H<sub>2</sub>O<sub>2</sub> (ref.<sup>24</sup>) (results given in brackets in this range without specification of the reagent). With specification of the reagent: SbCl<sub>3</sub> (ref.<sup>25</sup>), trichloroacetic acid-chloroamine T (TCE)<sup>26</sup>, fluorescein-bromine (detection of isolated double bonds, ref.<sup>27</sup>), UV fluorescence at 366 nm (UV), chlorine-benzidine (detection of NH, ref.<sup>12</sup>). The relative mobility ( $R_{x=100}$ ) related to a standard substance is given. Colours: blue (bl), brown (br), dark (d), yellow (y), green (gr), red (r), mauve (m), violet (v), orange (or), negative (neg.), positive (pos.), bright (bt). Pre-

parative layer chromatography (PLC) was done at analytical TLC plates (0.25 mm thickness) with loading up to 50 mg. Higher amounts (up to 200 mg) were separated at plates of 2 mm thickness (Merck). Detection: UV 254 and/or 366 nm or spraying H<sub>2</sub>O or I<sub>2</sub>- vapour contact. Elution: acetone. Corrected melting points (m.p.) were determined with the Boetius melting point microscope apparatus (VEB Analytik, Dresden). Solvent for crystallization is given in parentheses. To detect decomposition the melt was investigated by TLC. Ultraviolet spectra [ $\lambda_{\max}$  in nm (log  $\epsilon$ )] were recorded with the spectral-photometer DK-2A (Beckman Instruments Inc., Fullerton, U.S.A.) in ethanol. Infrared spectra (KBr, wavenumbers in cm<sup>-1</sup>) were recorded on Specord 75 IR (VEB Carl Zeiss, Jena, Germany) or Spectromom 2000 (MOM, Budapest, Hungary). Intensities: strong (st), very strong (vst), weak (w), medium (m), shoulder (sh), sharp (s), broad (br).

Electron impact mass spectra (MS,  $m/e$  is given) were recorded with a mass spectrometer MS 902 S (AEI, Manchester, G.B.). The composition of the important ions is secured by high resolution: resolution 10 000, 10% valley. Difference between found and calculated values maximal  $\pm 3$  millimasses. Electron energy: 70 eV. Source temperature is given at each compound. <sup>1</sup>H NMR spectra were measured on an NMR spectrometer KRH 100 (Central Scientific Instruments Construction, Academy of Sciences, Berlin, Germany) and BS 497 (TESLA, Brno, Czechoslovakia) at 100 MHz in the continuous wave mode in CDCl<sub>3</sub>. Chemical shifts in ppm ( $\delta$ -scale) were referred to internal TMS. Coupling constants ( $J$ ) were given in Hz. Aqueous solutions used: Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (5%), KHCO<sub>3</sub> (10%). Solutions in organic solvents were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. Pyridine was removed by washing with concentrated CuSO<sub>4</sub> solution. Solvents were removed using a rotatory evaporator at 13 mm Hg and low bath temperatures.

#### 17 $\beta$ -(5-Oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ -androst-14-en-3 $\beta$ -ol (**9**)

*First method:* A solution of **1** (250 mg, 0.92 mmol) in methanol (MeOH) (12 ml) was saturated with gaseous NH<sub>3</sub> at 0 °C. The mixture was heated in a steel autoclave at 110 °C for 17 h. The evaporation residue is crude<sup>2,3</sup> **5** (271 mg). To its solution in ethanol (27 ml), H<sub>2</sub>SO<sub>4</sub> (2%, 2.7 ml) was added and the mixture was heated to reflux for 2 h. The major part of ethanol was distilled off at 25 °C, to the residue chloroform was added and washed until neutral. PLC (b, 3  $\times$ ) yields **9** (77 mg, 33%, 0.22 mmol).

*Second method:* Crude **5** (100 mg, 0.27 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 ml), mixed with silica gel (5 g) and SbCl<sub>3</sub> (100 mg, 0.44 mmol) and heated at 120 °C for 20 min. Elution with acetone with 4% of pyridine, washing and PLC yields **9** (56 mg, 54%). TLC (b, 3  $\times$ ): R<sub>f</sub>: 58 (SbCl<sub>3</sub>: bl, *m*-DNB: r). UV:  $\leq 205$ . IR: 3 400 (sh), 3 250 (st, N-H), 1 715 (vst), 1 640 (vst, amide I), 1 600 (m,  $\Delta^{20(22)}$ ), 1 445 (st), 1 375 (w), 1 270 (st, amide III), 1 065, 1 040, 760, 700. MS (170 °C): 355 BP (M), 340 vst (M - CH<sub>3</sub>), 337 vst (M - H<sub>2</sub>O), 322 vst (M - H<sub>2</sub>O - CH<sub>3</sub>), 283 st (M - H<sub>2</sub>O - C<sub>4</sub>H<sub>6</sub>: ring A), 203 w, 110 (C<sub>6</sub>H<sub>8</sub>NO: butenamide ring + 17-CH + 16-CH<sub>2</sub> + H). <sup>1</sup>H NMR: 5.91 (s, 1 H, 22-H); 5.20 (m, 2 H, 15-H<sub>2</sub>); 4.12 (s, 1 H, 3 $\alpha$ -H); 3.95 (s, 2 H, 21-H<sub>2</sub>); 2.88 (m, 1 H, 17 $\alpha$ -H); 2.65 (s, NH); 1.02 (s, 3 H, 19-H<sub>3</sub>); 0.79 (s, 3 H, 18-H<sub>3</sub>).

#### 17 $\beta$ -(5-Oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ -androst-14-en-3 $\beta$ -yl Acetate (**11**)

Compound **4** (827 mg, 2.08 mmol) was dissolved in MeOH (200 ml) and saturated with gaseous NH<sub>3</sub> at 0 °C. Evaporation at room temperature after 5 days gave 992 mg residue. To its

solution in chloroform (50 ml),  $\text{SbCl}_3$  (992 mg, 4.35 mmol) and  $\text{SiO}_2$  (50 g) were added and the mixture was evaporated. Heating the residue at 120 °C for 20 min, elution with acetone with 4% of pyridine, evaporation, solution in chloroform, washing successively with dilute HCl,  $\text{KHCO}_3$  and water, evaporation. Column chromatography (silica gel 0.03–0.2 mm, Merck) with chloroform–acetone gave **11** (693 mg, 81%). TLC (c, 3 ×):  $R_F$  0.42;  $R_1$  89 (*m*-DNB: r). MS (170 °C): 411 (M), 393 (M –  $\text{H}_2\text{O}$ ), 110 ( $\text{C}_6\text{H}_8\text{ON}$ : butenamide ring + 17-CH + 16- $\text{CH}_2$  + H). The byproduct was **9** (59 mg, 8%) with properties as above.

17 $\beta$ -(1-Methyl-5-oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ -androst-14-en-3 $\beta$ -yl Acetate (**12**)

Compound **15** (500 mg, 1.26 mmol) was dissolved in pyridine (15 ml), methylamine (33% in water, 3.5 ml, 37.1 mmol) was added and after 2 days at room temperature the mixture was evaporated. To a solution of the residue (1.07 g) in pyridine (10 ml), acetic anhydride (10 ml) and, after 16 h, MeOH (6 ml) were added. One hour later the mixture was evaporated. The residue (1.123 g) was dissolved in chloroform (20 ml), silica gel (11 g) and  $\text{SbCl}_3$  (1.13 g, 4.96 mmol) were added and the solvent was removed. Treatment as above (second method) and column chromatography (silica gel 0.03–0.2 mm, Merck) with chloroform–acetone yields **12** (215 mg, 41%). TLC (c, 3 ×):  $R_F$  0.71,  $R_1$  120 ( $\text{SbCl}_3$ : bl, *m*-DNB: r, NH: neg.). MS (170 °C): 411 w (M), 396 st (M –  $\text{CH}_3$ ), 397 st (M –  $\text{H}_2\text{O}$ ), 378 vst (M –  $\text{H}_2\text{O}$  –  $\text{CH}_3$ ), 351 vst (M – AcOH), 297 st (M –  $\text{Ac}_2\text{O}$  –  $\text{C}_4\text{H}_6$ : ring A), 203 w, 124 ( $\text{C}_7\text{H}_{10}\text{NO}$ : N-Me-butenamide ring + 17-CH + 16- $\text{CH}_2$  + H).

14-Hydroxy-17 $\beta$ -[(2*R*)-2-hydroxy-5-oxo-2,5-dihydropyrrol-3-yl]-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**17**)

3,16-Diacetylgitoxigenin **15** (140 mg, 0.29 mmol) in 14 ml  $\text{CCl}_4$  (distilled from  $\text{P}_2\text{O}_5$ ) was brominated with *N*-bromosuccinimide (158 mg, 0.89 mmol) under reflux and illumination with a 250 W tungsten lamp for 20 min. Succinimide was filtered off and the filtrate was washed successively with  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{KHCO}_3$  and water, and evaporated. To a solution of the crude bromination product **16** in chloroform (14 ml), a solution of  $\text{NH}_3$  in MeOH (7.5%, 0.84 ml, 3.4 mmol) was added. After 25 h, the mixture was filtered and the filtrate was evaporated. PLC of the crude product (165 mg) gave **17** (75 mg, 51%). From chloroform–ether clusters of needles, m.p. 185–196 °C (decomp.: 3 less polar products). TLC (d, 2 ×):  $R_{15}$  41 ( $\text{SbCl}_3$ : bt-bl, *m*-DNB: neg., NH: pos.). UV: 212 (4.142). IR: 3 400 (st, br), 3 280 (sh, N-H), 1 740–1 680 (vst, br), 1 630 (m, sh), 1 445 < 1 380 (acetyl), 1 250–1 230 (vst, br), 1 150 (w), 1 090 (st), 1 025 (st), 700 (w). MS (180 °C): 471 (M –  $\text{H}_2\text{O}$ ), 453 (M – 2  $\text{H}_2\text{O}$ ), 429 (M – AcOH), 411 (M – AcOH –  $\text{H}_2\text{O}$ ).  $^1\text{H}$  NMR: 6.74 (s, NH, disappeared in  $\text{D}_2\text{O}$ ); 6.17 (s, 1 H, 22-H); 5.28 (m, 1 H, 16 $\alpha$ -H); 5.26 (s, 1 H, 21 $\alpha$ -H); 5.12 (s, 1 H, 3 $\alpha$ -H); 3.23 (d, 1 H, 17 $\alpha$ -H); 2.15 (q, 1 H, 15 $\alpha$ -H); 2.07 (s, 3 H, 3 $\beta$ -OAc); 2.04 (s, 3 H, 16 $\beta$ -OAc); 0.99 (s, 1 H, 19- $\text{CH}_3$ ); 0.95 (s, 3 H, 18- $\text{CH}_3$ ).

14-Hydroxy-17 $\beta$ -[(2*R*)-2-hydroxy-1-methyl-5-oxo-2,5-dihydropyrrol-3-yl]-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**18**)

To a solution of the crude bromination product of **15** (663 mg, 1.40 mmol) in chloroform (2 ml) and dioxane (8 ml), a solution of methylamine (33% in water, 0.61 ml, 6.47 mmol) was added and the mixture was evaporated after 15 h. PLC (d, 3 ×) gave **18** (404 mg, 60%). From chloroform–ethanol columns with inclined ends, m.p. 146–152 °C. TLC (d, 2 ×):  $R_{15}$

52,  $R_{17}$  205 (SbCl<sub>3</sub>: y, *m*-DNB: neg., NH: neg.). UV: 211 (4.061). IR: 3 450 (st, br), 1 740–1 690 (vst, br), 1 630 (m, sh), 1 450 (N-Me, in comparison to **17** considerably increased), 1 450 < 1 385 (acetyl), 1 255–1230 (vst, br), 1 158 (w), 1 098 (st), 1 030 (st), 710 (w). MS (160 °C): 485 st (M - H<sub>2</sub>O), 467 (M - 2 H<sub>2</sub>O), 443 (M - AcOH), 425 BP (M - AcOH - H<sub>2</sub>O), 410 st (M - AcOH - H<sub>2</sub>O - CH<sub>3</sub>), 365 vst (M - 2 AcOH - H<sub>2</sub>O), 350 (M - 2 AcOH - H<sub>2</sub>O - CH<sub>3</sub>), 314 vst (M - H<sub>2</sub>O - AcOH - lactam ring), 203 st (C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>: ring D-Δ<sup>14,16</sup> + 12-CH<sub>2</sub> + 18-CH<sub>3</sub> + 21-OH,N-CH<sub>3</sub>-butenamide ring), 113 (C<sub>5</sub>H<sub>7</sub>NO<sub>2</sub>: 21-OH,N-CH<sub>3</sub>-butenamide ring + H), 95 (113 - H<sub>2</sub>O). <sup>1</sup>H NMR: 7.54 (d, *J* = 12, 21-OH, + D<sub>2</sub>O: extinguished); 6.17 (s, 1 H, 22-H); 5.18 (tr, 1 H, 16α-H); 5.10 (s, 1 H, 3α-H); 4.82 (d, *J* = 11, 1 H, 21α-H, + D<sub>2</sub>O: 4.87 s); 3.47 (q, 1 H, assignment unclear); 3.20 (d, 1 H, 17α-H); 2.94 (s, 3 H, N-CH<sub>3</sub>); 2.62 (q, 1 H, 15α-H); 2.04 (s, 3 H, 3β-OAc); 1.99 (s, 3 H, 16β-OAc); 0.88 (s, 3 H, 18-H<sub>3</sub>); 0.57 (s, 3 H, 19-H<sub>3</sub>).

14,2'-Epoxy-17β-[(2'*S*)-1'-methyl-5'-oxo-2',5'-dihydropyrrol-3'-yl]-5β,14β-androstane-3β,16β-diyl Diacetate (**23**)

A solution of **18** (100 mg, 0.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (9 ml) was cooled to -70 °C and the mixture of CH<sub>2</sub>Cl<sub>2</sub> (0.84 ml), SOCl<sub>2</sub> (0.15 ml, 1.18 mmol) and DMF (0.01 ml) was added. After 4.5 h at 22 °C the mixture was washed until neutral. PLC (a, 2 ×) of the residue gave **23** (68.5 mg, 71%). Clusters of fine needles (acetone), m.p. 236–238 °C (decomp.). TLC (a, 2 ×):  $R_{15}$  151,  $R_7$  83,  $R_{18}$  490 (SbCl<sub>3</sub>: y, *m*-DNB: neg., NH: neg., Δ: neg.). UV: 211 (4.080). IR: 1 738 (vst), 1 705 (vst), 1 690 (vst, br), 1 450 < 1 388 (acetyl), 1 255 (vst, br), 1 150 (w), 1 070 (st), 1 025 (st), 880 (m), 698 (w). MS (175 °C): 485 (M), 467 (M - H<sub>2</sub>O), 443 (M - ketene), 425 BP (M - AcOH), 365 vst (M - 2 AcOH), 350 (M - 2 AcOH - CH<sub>3</sub>), 314 vst (M - lactam ring - 14-O - AcOH), 203 s (C<sub>15</sub>H<sub>23</sub>: rings A, B, C - AcOH - C<sub>14</sub>, and C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub> [cf. MS of **18**] 1 : 2).

14-Hydroxy-17β-[(2*R*)-1-methyl-5-oxo-2-(tosyloxy)-2,5-dihydropyrrol-3-yl]-5β,14β-androstane-3β,16β-diyl Diacetate (**22**)

Compound **18** (from 500 mg **15**, 1.05 mmol) was dissolved in pyridine (2 ml) cooled to -70 °C and tosyl chloride (2.43 g, 1.28 mmol, in 2.5 ml pyridine) was added. After 15 h at room temperature MeOH (2 ml) was added and after 30 min all solvents were removed. PLC (b, 2 ×) gave **22** (230 mg, 29% from **15**, 48% from **18**). Short prisms from acetone-pentane, m.p. 205–209 °C (decomp.). TLC (b, 2 ×):  $R_{18}$  610,  $R_{15}$  137 (SbCl<sub>3</sub>: y, *m*-DNB: neg., NH: neg.). UV: 220 (4.216). IR: 3 250 (br), 1 730 (st), 1 700 (vst), 1 590 (m), 1 445, 1 380, 1 245–1 225 (vst, br), 1 070, 1 020; O-Ts: 1 565 (w), 1 320 (st), 1 175 (vst), 1 110 (m), 815 (m), 735 (m), 650 (m). MS (160 °C): 501 (M - C<sub>7</sub>H<sub>7</sub>OS), 441 st (501 - AcOH), 425 vst (M - C<sub>7</sub>H<sub>7</sub>O<sub>2</sub>S - AcOH), 381 vst (501 - 2 AcOH), 314 vst (M - lactame ring - AcOH - H<sub>2</sub>O), 265 st (M - TsOH - 2 AcOH), 203 BP (C<sub>15</sub>H<sub>23</sub>: rings A, B, C - AcOH - C<sub>14</sub>), 155 (C<sub>7</sub>H<sub>7</sub>O<sub>2</sub>S: Ts), 139 (C<sub>7</sub>H<sub>7</sub>OS), 91 (C<sub>7</sub>H<sub>7</sub>: tropylium ion). <sup>1</sup>H NMR: 7.74–7.92 (m, Ar-H); 7.26–7.40 (m, Ar-H); 7.00 (s, 3 H, N-CH<sub>3</sub>); 6.60 (s, 1 H, 22-H); 5.45 (tr, 1 H, 16α-H); 5.15 (s, 1 H, 3α-H); 4.76 (m, 1 H, 21α-H, + D<sub>2</sub>O: s); 3.27 (d, 1 H, 17α-H); 2.44 (s, 3 H, Ar-CH<sub>3</sub>); 2.05 (s, 3 H, 3β-OAc); 1.96 (s, 3 H, 16β-OAc); 0.98 (s, 1 H, 19-H<sub>3</sub>); 0.90 and 0.87 (s, 3 H, 18-H<sub>3</sub>).

14-Hydroxy-17 $\beta$ -(1-methyl-5-oxo-2,5-dihydropyrrol-3-yl)-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**24**) and 14-Hydroxy-17 $\beta$ -[1-methyl-5-oxotetrahydropyrrol-3 $\xi$ -yl]-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**25**)

Crude **22** (from 500 mg gitoxigenin (deacetyl-**15**), 1.28 mmol) was dissolved in MeOH (2 ml) and after addition of AcOH (1 ml), H<sub>2</sub>O (0.1 ml) and Zn dust (1 g) shaken for 11.5 h. The evaporation residue of the filtrate was suspended in chloroform and washed. PLC (e, 3  $\times$ ) of the residue gave **24** (45 mg, 12% from **15**, 43% from **22**) and **25** (64 mg, 17% from **15**). Compound **24**: C<sub>26</sub>H<sub>41</sub>NO<sub>6</sub> (487.63), crystal warts (acetone–diethyl ether–pentane), m.p. 198–203 °C. TLC (e, 2  $\times$ ): R<sub>15</sub> 74, R<sub>18</sub> 122 (SbCl<sub>3</sub>; UV br-bl, day light: green, *m*-DNB: r-v, NH: neg.). UV: 207. IR: 3 250 (st), 1 735 (vst), 1 670\* (vst), 1 620 (sh, m), 1 477, 1 370, 1 405\* (w), 1 250–1 230 (vst, br), 1 100 (m), 1 030 (st), 735\* (w) (\* means additional bands compared to **15**). MS (170 °C): 487 st (M), 427 vst (M – AcOH), 409 vst (M – AcOH – H<sub>2</sub>O), 367 st (M – 2 AcOH), 349 (M – 2 AcOH – H<sub>2</sub>O), 203 st (C<sub>15</sub>H<sub>23</sub>; rings A, B, C – AcOH – C<sub>14</sub>), 196 vst (C<sub>10</sub>H<sub>14</sub>O<sub>3</sub>N: N-Me-butenamide ring + 17-CH + 16-CH-OAc + 15-CH<sub>2</sub> + H), 141 st (C<sub>11</sub>H<sub>13</sub>O<sub>2</sub>N:  $\Delta^{16}$ -ring-D + 14-OH + N-Me-butenamide ring + 18-CH<sub>3</sub>), 137 BP (C<sub>8</sub>H<sub>10</sub>NO: 196 + H – AcOH). <sup>1</sup>H NMR: 5.52 (tr, 1 H, 16 $\alpha$ -H); 5.10 (s, 1 H, 3 $\alpha$ -H); 4.83 (s, 1 H, 22-H); 3.87 (s, 2 H, 21-H<sub>2</sub>); 3.06 (s, 3 H, N-CH<sub>3</sub>); 2.65 (q, 1 H, 15 $\alpha$ -H); 2.20 (d, 1 H, 17 $\alpha$ -H); 2.04 (s, 3 H, 3 $\beta$ -OAc); 1.88 (s, 3 H, 16 $\beta$ -OAc); 0.97 (s, 3 H, 19-H<sub>3</sub>); 0.89 (s, 3 H, 18-H<sub>3</sub>).

14-Hydroxy-17 $\beta$ -(2'-maleimidoyl)-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**26**)

Compound **17** (500 mg, 1.02 mmol) was dissolved in chloroform (5 ml) and after addition of active MnO<sub>2</sub> (5 g, 57.5 mmol) was shaken for 1 h. MnO<sub>2</sub> was filtered off and washed with chloroform. PLC (d, 3  $\times$ ) gave **26** (360 mg, 72%). Thick needles (acetone–hexane), m.p. 270–273 °C (decomp.: about 50% of a more polar compound). TLC (e, 2  $\times$ ): R<sub>17</sub> 240, R<sub>15</sub> 87 (y-or, neg., NH: pos.). IR: 3 510 (st, s), 1 710 (vst, br), 1 615 (m), 1 440 < 1 375 (st), 1 245 (vst), 1 080, 1 015 (st), 880, 685; N-H: 3 150 (st, br), 3 045 (m). MS (190 °C): 487 (M), 427 (M – AcOH), 409 (M – AcOH – H<sub>2</sub>O), 367 (M – 2 AcOH), 263 (203 + AcOH), 241 (C<sub>15</sub>H<sub>15</sub>NO<sub>2</sub>; rings C +  $\Delta^{14,16}$ -D + maleimide ring + 7-CH<sub>2</sub> + 13 $\beta$ -CH<sub>3</sub>), 203 BP (C<sub>15</sub>H<sub>23</sub>), 191 (C<sub>10</sub>H<sub>9</sub>NO<sub>3</sub>;  $\Delta^{16}$ -ring D + 14-OH + 13 $\beta$ -CH<sub>3</sub> + maleimide ring).

14-Hydroxy-17 $\beta$ -(*N*-methylmaleinimidoyl)-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**27**)

Compound **18** (500 mg, 0.99 mmol) was oxidised with MnO<sub>2</sub> for 4 h (5 g, 57.5 mmol) as above. PLC (d, 3  $\times$ ) yields **27** (351 mg, 70%). M.p. 172–177 °C (chloroform–ethanol 9 : 1). TLC (e, 2  $\times$ ): R<sub>18</sub> 145, R<sub>15</sub> 107 (or, neg., NH: neg.). IR: 3 450 (sh), 1 730 (vst), 1 700 (vst), 1 620 w, 1 445, 1 375 (st), 1 245 (vst), 1 080, 1 015 (st), 880, 685; N-H: 3 150 (st, br), 3 045 (m). MS (175 °C): 501 st (M), 441 vst (M – AcOH), 381 (M – 2 AcOH), 363 (M – 2 AcOH – H<sub>2</sub>O), 348 (M – 2 AcOH – H<sub>2</sub>O – CH<sub>3</sub>), 314 st (M – AcOH – H<sub>2</sub>O – *N*-methylmaleimide ring), 263 (203 + AcOH), 205 (C<sub>11</sub>H<sub>11</sub>NO<sub>3</sub>;  $\Delta^{16}$ -ring D + 14-OH + *N*-methylmaleimide ring + 13 $\beta$ -CH<sub>3</sub>) 203 BP (C<sub>15</sub>H<sub>23</sub>).

Reaction of **26/27** with H<sub>2</sub>S

Compound **26** (10 mg, 0.02 mmol) was dissolved in pyridine (2 ml) and saturated with H<sub>2</sub>S at 0 °C. TLC (d, 2  $\times$ ) shows that after 24 h **26** was transformed mainly (90%) to a more polar product which contains S<sup>2+</sup>: R<sub>26</sub> 77 (H<sub>3</sub>PO<sub>4</sub>; y, iodine–azide–starch reagent<sup>28</sup> for S<sup>2+</sup>: pos.).



Compound **27** reacts analogously but somewhat faster: after 15 h 100% transformation.  $R_{27}$  14 (y,  $S^{2+}$ : pos.).

3 $\beta$ -[3,4-Di-*O*-acetyl-2,6-dideoxy- $\beta$ -D-*ribo*-hexopyranosyl-(1 $\rightarrow$ 4)-3-*O*-acetyl-2,6-dideoxy- $\beta$ -D-*ribo*-hexopyranosyl-(1 $\rightarrow$ 4)-3-*O*-acetyl-2,6-dideoxy- $\beta$ -D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-17 $\beta$ -(*N*-methylmaleimidoyl)-5 $\beta$ ,14 $\beta$ -androstane-16 $\beta$ -yl Acetate (**30**)

Penta-*O*-acetylgitoxin **28** (2 g, 2.02 mmol) in  $\text{CCl}_4$  (200 ml) was brominated with *N*-bromosuccinimide (1.44 g, 8.09 mmol) according to the standard procedure (see preparation of **17**). The reaction mixture was filtered and the filtrate was washed successively with  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{KHCO}_3$ , water and dried. Methylamine (2 ml, 33% in ethanol, 21.2 mmol) was added to the solution of the bromination product. After 20 h at room temperature the mixture was washed successively with 0.1 M HCl,  $\text{KHCO}_3$  and water, dried and evaporated *in vacuo*. The oily residue obtained was chromatographed on silica gel (100 g). Elution with chloroform-ethanol (98 : 2) gave the 21-hydroxy-*N*-methylactam **29** (627 mg, 30.4%) which was dried over  $\text{P}_2\text{O}_5$  *in vacuo*, dissolved in chloroform (20 ml) and shaken with active  $\text{MnO}_2$  (6.8 g, 78.2 mmol) for 2 h. The reaction product was purified on silica gel (30 g). Elution with chloroform provided **30** (527 mg, 84.2% from **29**) which crystallized from ether-methanol-pentane as needles, m.p. 156–159 °C. MS: 631 ( $\text{C}_{34}\text{H}_{49}\text{NO}_{10}$ :  $\text{M}^+$  - 3'',3''',4''''-tri-*O*-acetyl(digitoxosyl)<sub>2</sub>), 613 (631 -  $\text{H}_2\text{O}$ ), 571 (631 -  $\text{CH}_3\text{COOH}$ ), 553 (613 -  $\text{CH}_3\text{COOH}$ ), 511 (631 - 2  $\text{CH}_3\text{COOH}$ ), 493 (613 - 2  $\text{CH}_3\text{COOH}$ ), 459 (aglycon:  $\text{M}^+$  - 3',3'',3''',4''''-tetra-*O*-acetyl(digitoxosyl)<sub>3</sub>), 442 (459 - OH), 399 (459 -  $\text{CH}_3\text{COOH}$ ), 387 ( $\text{C}_{18}\text{H}_{27}\text{O}_9$ : 3'',3''',4''''-tri-*O*-acetyl(digitoxosyl)<sub>2</sub>), 382 (442 -  $\text{CH}_3\text{COOH}$ ), 327 (387 -  $\text{CH}_3\text{COOH}$ ), 231 ( $\text{C}_{10}\text{H}_{15}\text{O}_6$ : 3''',4''''-di-*O*-acetyldigitoxosyloxy), 215 ( $\text{C}_{10}\text{H}_{15}\text{O}_5$ : 3''',4''''-di-*O*-acetyldigitoxosyl), 155 (215 -  $\text{CH}_3\text{COOH}$ ), 95 (215 - 2  $\text{CH}_3\text{COOH}$ ). MS (high resolution): 459.2618 (calculated for  $\text{C}_{26}\text{H}_{37}\text{NO}_6$ : 459.2621), 399.2397 (calculated for  $\text{C}_{24}\text{H}_{33}\text{NO}_4$ : 399.2409).

14-Hydroxy-17 $\beta$ -(1,2-*N*-methylcarbamoylviny)-5 $\beta$ ,14 $\beta$ -androstane-3 $\beta$ ,16 $\beta$ -diyl Diacetate (**20**)

Compound **15** (250 mg, 0.53 mmol) was brominated with NBS (281 mg, 1.58 mmol) by the standard procedure. The crude reaction product (containing besides the 21-bromo derivative **16** the 21,21-dibromo derivative **19**) was dissolved in chloroform (27 ml), methylamine (33% in  $\text{H}_2\text{O}$ , 0.23 ml, 2.44 mmol) was added and the mixture was shaken for 10 min. PLC (f) and elution of the zone  $R_F$  0.21 yields **20** (25 mg, 23% from **15**). Clusters of fine needles (chloroform-diethyl ether), m.p. 217–222 °C. TLC (e, 2  $\times$ ):  $R_{15}$  19 (SbCl<sub>3</sub>: y, neg., NH: pos.). UV: 218 (4.087). IR: 3 400 (st, br), 3 300 (sh, N-H), 1 735 (vst), 1 635 (vst, amide I), 1 535 (st, amide II), 1 455 (w, N-Me), 1 380, 1 370 (m, C-N), 1 255–1 230 (vst, br), 1 155 (w), 1 025 (m, C-N). MS (160 °C): 532 w (M), 501 st (M -  $\text{CH}_3\text{NH}_2$ ), 472 (M - AcOH), 441 vst (M - AcOH -  $\text{CH}_3\text{NH}_2$ ), 400 (M - 2  $\text{CONHCH}_3$  -  $\text{CH}_3$  - H), 381 st (M - 2 AcOH -  $\text{CH}_3$  -  $\text{NH}_2$ ), 263 (203 + AcOH), 203 BP ( $\text{C}_{15}\text{H}_{23}$ ). <sup>1</sup>H NMR: 6.22 (s, 1 H, 22-H); 5.37 (tr, 1 H, 16 $\alpha$ -H); 5.11 (s, 1 H, 3 $\alpha$ -H); 3.02 (d, 1 H, 17 $\alpha$ -H); 2.80 and 2.85 (2 d, 2  $\times$  3 H, 2  $\times$  N-CH<sub>3</sub>, + D<sub>2</sub>O: one s); 2.52 (q, 1 H, 15 $\alpha$ -H); 2.12 (s, 3 H, 3 $\beta$ -OAc); 1.88 (s, 3 H, 16 $\beta$ -OAc); 1.06 (s, 3 H, 18-H<sub>3</sub>); 0.98 (s, 3 H, 19-H<sub>3</sub>). For  $\text{C}_{29}\text{H}_{44}\text{NO}_7$  (532.7) calculated: 65.39% C, 8.29% H, 5.21% N; found: 65.37% C, 8.31% H, 5.23% N.

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