Synthetic Cardenolides and Related Products. III.[1] Isocardenolides[2]

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The synthesis of novel cardenolides, closely related to naturally-occurring cardiotonic agents, is described. They were obtained from 17β-[3-furyl]androstanes derived from digitoxigenin, gitoxigenin, digoxigenin, and strophanthidin by hypohalous acid or organic peracid oxidation. The preparation of β-D- and α-L-glycosidic derivatives is also reported.

On a synthétisé de nouveaux agents cardiotoniques, isomères des cardénolides naturels. Ils ont été obtenus par l'oxidation à l'aide de l'acide hypohaleux ou des peracides organiques de [3-furyl]-17β androstanes, dérivés de la digitoxigénine, la digoxigénine, la gitoxigénine et la strophanthidine. Des dérivés β-D- et α-L-glycosidiques sont aussi décrits.

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As part of a program aimed to improve the therapeutic ratio of cardiotonic agents, we prepared novel isocardenolides,[3] which possessed all the characteristics of the natural cardenolides except for the location of the oxo group of the 5-membered lactone (3). In addition, 21-hydroxydigitoxigenin was also synthesized.

When 17β-[3-furyl]-5β-androstane-3β,14β-diol 3-acetate (**2***b*) was treated with a hypohalous acid in aqueous dioxane, lactones **3***a* and **4** were isolated. The major product was **3***a* when 1 equiv. of the oxidant was used, while a larger excess of the reagent (2 equiv.) afforded mainly lactone **4**. The structures were ascertained by spectral analyses (see Experimental). Furthermore, the aldehyde **4** was converted to the acid **5** and to the corresponding saturated lactone-aldehyde **6** by reducing the ene-dione system with zinc and acetic acid. Finally, **3***a* and **4** were both oxidized with potassium permanganate to the well-known (5) α-ketolactone **7**. This constitutes an unambiguous proof of the 17β-stereochemistry of lactone **3***a* (Scheme 1).

Scheme 2 illustrates a likely mechanism for the formation of **3***a* and **4**. Initially an electrophilic attack of X^+ of the hypohalous acid on the less-hindered side of the furan ring, concerted with a nucleophilic addition of OH^-, affords **9**. Elimination of HX, followed by rearrangement of the resulting hydroxyfuran **10**, yields **3***a*. In a competitive pathway further oxidation of **9** gives the bromolactone[4] **11**, which is converted into **4** by an internal attack of the 14-hydroxyl on the carboxyl group with concomitant elimination of HX.

The furyl derivatives (**2**) were in general obtained by diisobutylaluminum hydride reduction of the natural cardenolides (**1**) (6). An alternative process for the preparation of **2***a* was also used. Reduction of digitoxigenin **1***a* with an excess diisobutylaluminum hydride afforded the diol **8**, which was oxidized to **2***a* with 1 equiv. triethylamine – sulfur trioxide complex (7) (Scheme 1). In this last process the key step involves the formation of an unstable lactol **13**, which upon elimination of water affords the furan ring. This lactol originates from the cyclization of the hydroxyaldehyde **12** resulting from the oxidation of the diol **8** (Scheme 3).

In Scheme 4 are outlined the furan derivatives (**2**) and the isomeric cardenolides (**3**) that were prepared from digitoxigenin (**1***a*), digoxin (**1***b*), digoxigenin (**1***c*), and gitoxigenin (**1***d*). During the hypohalous acid oxidation of the furan, the 3-alcohols had to be protected as esters. In order to be able to regenerate the 3-hydroxy derivatives **3***c*, it was preferable to use chloroacetates rather than acetates. The latter could not be hydrolyzed under conditions (Na_2CO_3 or NaOH) which would prevent the opening of the lactone. On the other hand the chloroacetates were readily removed with potassium bicarbonate.

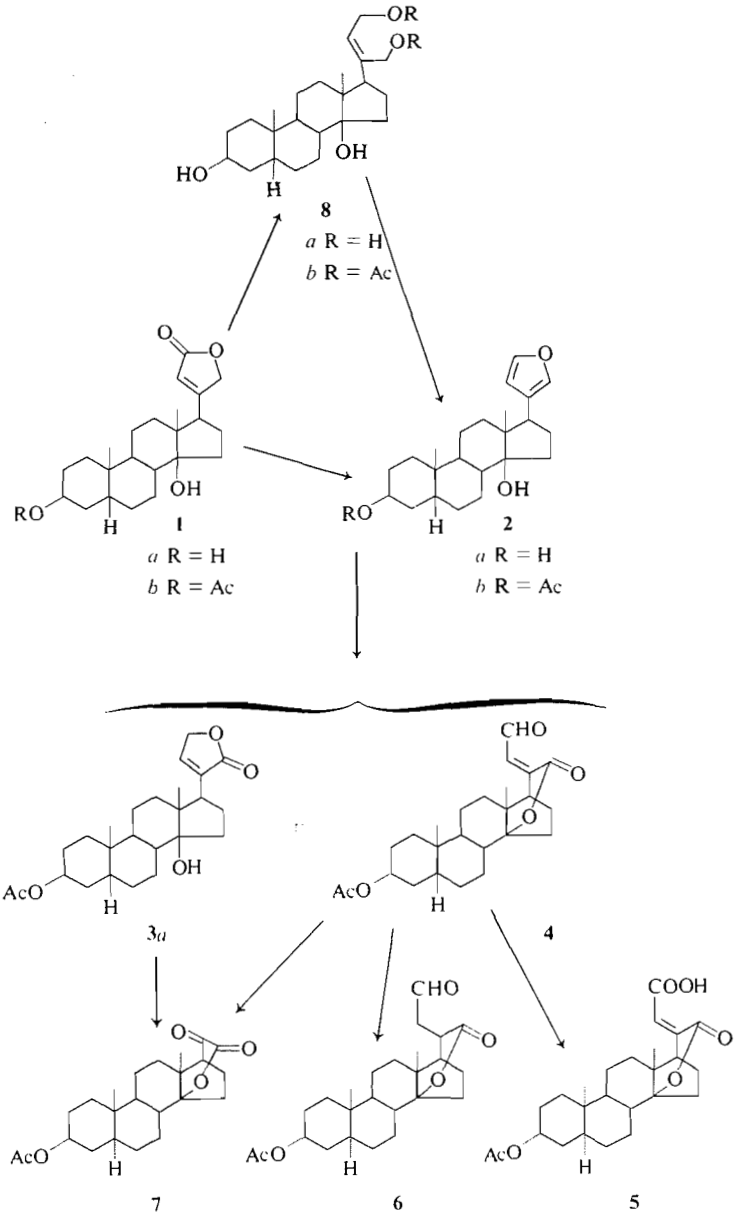
Since it is a well-established fact that cardiac

[1]For Parts I and II see refs. 1*a* and *b*.

[2]This work was presented in part at the 6th International Symposium on the Chemistry of Natural Products, Mexico, April 22, 1969 (2*a*).

[3]Other types of isocardenolides and modified butenolides have been reported in the literature (4).

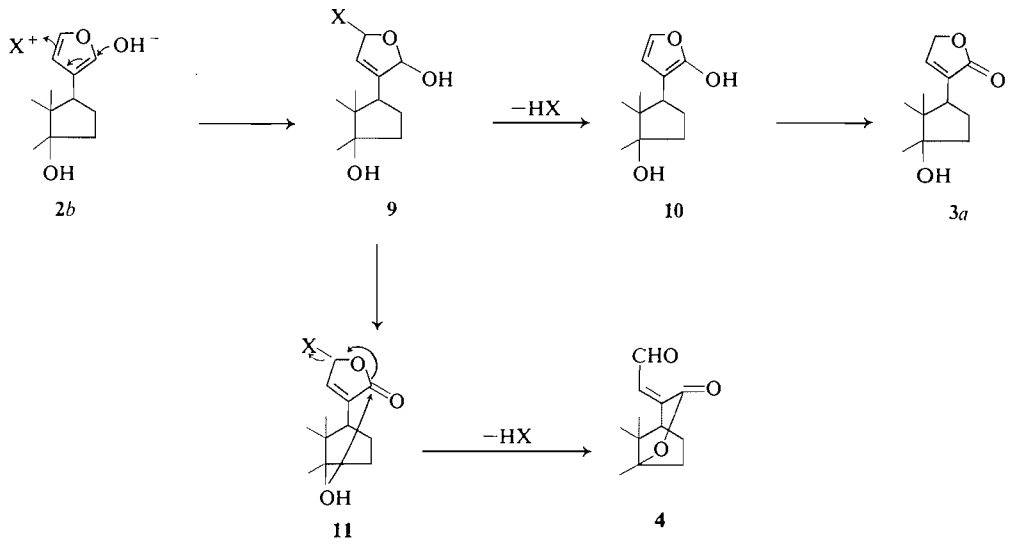
[4]Oxidation of 17β-[3-furyl] steroids in C/D *trans* series has yielded similar halolactones (2*b*).



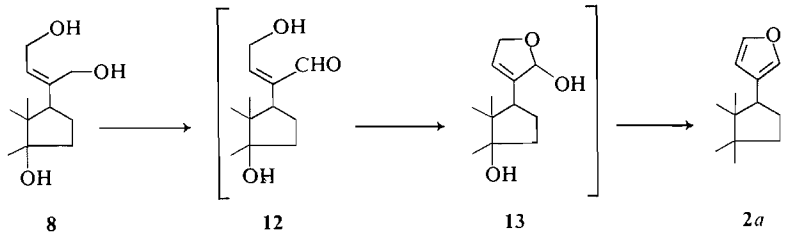
SCHEME 1

glycosides have greater therapeutic applications than the cardiac aglycones (9), it was obvious that our modified cardenolides should be converted into β-D- and α-L-pyranyloxy derivatives by a modified Koenigs–Knorr synthesis. Accordingly **3***c* and **3***m* were condensed with 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (12) to give as expected (10, 11) the acetylated β-D-glucopyranosyl derivatives **3***d* and **3***n*, and the orthoester **3***f* (characterized only in this case). Alcohol **3***c* was also condensed with 2,3,4-tribenzoyl-α-L-rhamnosyl bromide (13) to give, with net retention of configuration (10, 14), the α-L-rhamnopyranosyloxy analog **3***g*. The protecting esters were hydrolyzed as described by Zorbach *et al.* (15).

In a similar sequence of reactions digitoxin **1***b* was reduced by diisobutylaluminum hydride to afford **2***d*. Prior to the hypohalous acid oxidation



SCHEME 2



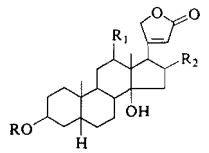
SCHEME 3

the hydroxyl groups of the sugar portion were protected as acetates. Regeneration of the alcohols was effected by mild hydrolysis with potassium bicarbonate at 37° for 15 days (17).

Finally a slightly different process had to be used to obtain the isocardenolide analog **19***b* of strophanthidin **14**. The 3-tetrahydropyranyl ether **15** (18) was reduced with diisobutylaluminum hydride to give the 17β-[3-furyl]-19-alcohol (**16***a*) (identified as the 3,19-diacetate, **16***b*), which was oxidized with manganese dioxide (8) to the aldehyde **17**. Removal of the pyranyl ether and chloroacetylation afforded **18** which was converted in the usual manner to the isocardenolide **19***a*. The 3-alcohol **19***b* as well as the pyranosyl derivatives **19***c* and **19***d* were obtained as previously described (Scheme 5).

The anomeric configurations of the pyranosyloxy isocardenolides **3***e*, **3***h*, **3***o*, and **19***d* were confirmed by application of the Klyne rule of molecular rotational additivity (16).

In order to obtain other modified lactones, **2***b* was oxidized with organic peracides to yield lactones **20** and **21**. The proportion of each was dependant on the experimental conditions. For example, lactone **20** was the major component when the solvent was acetone while in acetic acid **21** was predominant. The structures were again ascertained by spectral analysis. Furthermore potassium permanganate oxidation of both lactones afforded the known ketolactone **7**. Finally, reduction of **20** with sodium borohydride followed by acid treatment yielded digitoxigenin **1***b*. This constitutes an unequivocal proof of the 17β-stereochemistry of **20** and a novel synthesis of natural cardenolides (Scheme 6).



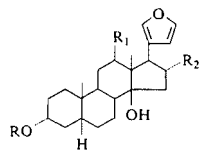
1

a R = R_1 = R_2 = H (digitoxigenin)

b R = Tridigitoxosyloxy; R_1 = R_2 = H (digitoxin)

c R = R_2 = H; R_1 = OH (digoxigenin)

d R = R_1 = H; R_2 = OH (gitoxigenin)



2

a R = R_1 = R_2 = H

b R = CCH_3; R_1 = R_2 = H

c R = CCH_2Cl; R_1 = R_2 = H

d R = Tridigitoxosyloxy; R_1 = R_2 = H

e R = Tridigitoxosyloxy tetraacetate; R_1 = R_2 = H

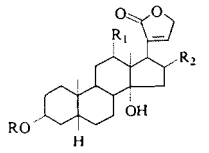
f R = R_2 = H; R_1 = OH

g R = CCH_3; R_1 = $OCCH_3$; R_2 = H

h R = CCH_2Cl; R_1 = $OCCH_2Cl$; R_2 = H

i R = R_1 = H; R_2 = OH

j R = CCH_3; R_2 = $OCCH_3$; R_1 = H



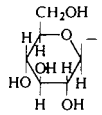
3

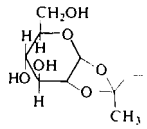
a R = CCH_3; R_1 = R_2 = H

b R = CCH_2Cl; R_1 = R_2 = H

c R = R_1 = R_2 = H

d R = β-D-Tetraacetylglucopyranosyloxy; R_1 = R_2 = H

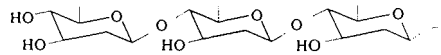
e R =  R_1 = R_2 = H

f R =  R_1 = R_2 = H

g R = α-L-Tribenzoylrhamnopyranosyloxy; R_1 = R_2 = H

h R =  R_1 = R_2 = H

i R = Tridigitoxosyloxy tetraacetate; R_1 = R_2 = H

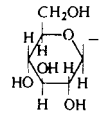
j R = 

k R = CCH_3; R_1 = $OCCH_3$; R_2 = H

l R = CCH_2Cl; R_1 = $OCCH_2Cl$; R_2 = H

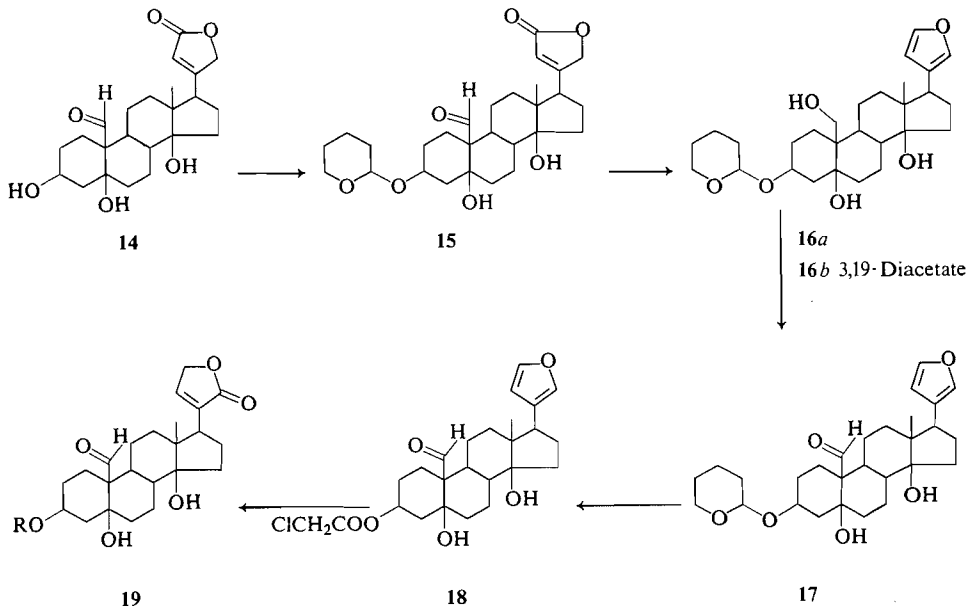
m R = R_2 = H; R_1 = OH

n R = β-D-Tetraacetylglucopyranosyloxy; R_2 = H; R_1 = OH

o R =  R_1 = OH; R_2 = H

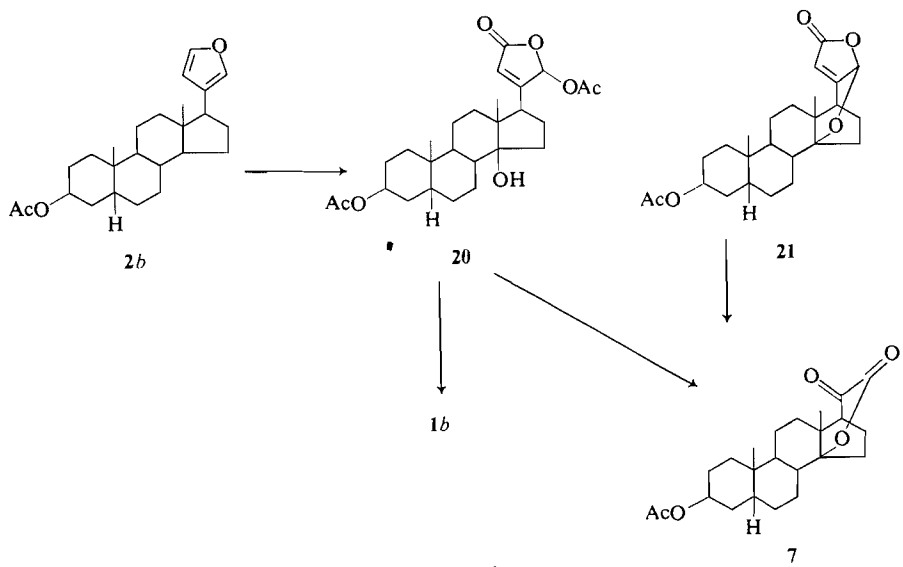
p R = CCH_3; R_2 = $OCCH_3$; R_1 = H

SCHEME 4



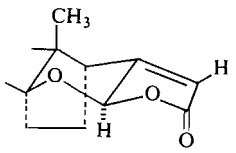
a R = $ClCH_2CO$
b R = H
c R = Tetraacetyl-β-D-glucopyranosyloxy
d R = β-D-Glucopyranosyloxy

SCHEME 5

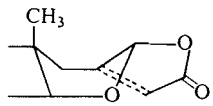


SCHEME 6

Although two isomers of lactone **21** may exist (**22** and **23**), we assign structure **22** to the isolated compound on conformational grounds.



22



23

Some of the results of the biological evaluation of our modified cardenolides have already been published (19) and will be described completely elsewhere.

Experimental

Melting points are uncorrected. The i.r. spectra were taken on a Perkin-Elmer 225 i.r. spectrophotometer and n.m.r. were recorded on a Varian-A-60A spectrometer operating at 60 MHz; TMS was used as internal standard. Merck silica gel 60 (60–230 mesh) was used. The $[\alpha]_D$ were taken at 20–23° in 1% chloroform solutions. Otherwise, the solvent is mentioned.

17β-[3-Furyl] Derivatives

Diisobutylaluminum Hydride Reduction

The reduction of the cardenolides was carried out by the slightly modified method of Minata and Nagasaki (6). The following example will illustrate the procedure.

17β-[3-Furyl]-3β-tetrahydropyranyloxy-5β,14β-androstane-5,14,19-triol (16a)

A 27% solution of diisobutylaluminum hydride in hexane (20 ml) diluted with dry THF (20 ml) was added dropwise, over a period of 5 min, under nitrogen, to a stirred solution at −20° of strophanthidin THP ether (**20**) (3.15 g) in dry THF (130 ml). The reaction mixture was stirred at the same temperature for 30 more min and allowed to warm up to 0° while a saturated solution of sodium potassium tartrate was added dropwise. After stirring for 15 min at room temperature, the mixture was filtered and the salts washed with ethyl acetate. The filtrate was washed to neutrality (salt saturated water), dried, and evaporated. The crude product was chromatographed on silica gel to give the title product (1.9 g).

In a similar way reduction of digitoxin, digitoxigenin, digoxigenin, and gitoxigenin gave **2***d*, **2***a*, **2***f*, and **2***i*, respectively.

Esters

17β-[3-Furyl]-5β,14β-androstane-3β,5,14,19-tetrol 3,19-Diacetate (16b)

A solution of the 3-THP ether **16***a* (0.100 g) in ethanol (3 ml) and 0.1 *N* hydrochloric acid (0.1 ml) was stirred at 45–50° for 4 h. The mixture was then concentrated under reduced pressure to half its original volume and poured into ice cold water. It was extracted with ethyl acetate and the organic solution washed with salt saturated water, dried, and evaporated (0.080 g).

The crude alcohol was acetylated in pyridine (1 ml) and acetic anhydride (0.5 ml) at room temperature overnight. The solution was poured into ice cold water and extracted with ethyl acetate. The organic solution was washed to neutrality, dried, and evaporated to give the title product. It was crystallized twice from methylene chloride – ether (0.057 g), m.p. 178–180°, $[\alpha]_D$ +41.6°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.44; H, 8.09.

The i.r. ν_{max}($CHCl_3$): 3570, 1720, and 865 cm^{-1}; n.m.r. ($CDCl_3$): 0.73 (C-18, CH_3), 2.06 and 2.11 (3- and 19-OAc), 3.20 (OH), 4.45 (C-19,—CH_2—O), 6.50 (C-4, H-furyl) 7.27 (C-5, H-furyl), and 7.36 p.p.m. (C-2, H-furyl).

In a similar way acetylation of **2***a*, **2***f*, **2***i*, and **2***d* gave, respectively, after chromatography:

2*b*. Yield 60% from **1***a*, methanol–water, m.p. 155–157°, $[\alpha]_D$ +17.5°.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.61; H, 8.65.

2*g*. Yield 35% from **1***c*, methanol–water, m.p. 158–161°, $[\alpha]_D$ +35.2°.

Anal. Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.42; H, 8.07.

2*j*. Yield 35% from **1***d*, methylene chloride – ether, m.p. 170–172°, $[\alpha]_D$ −14.9°.

Anal. Calcd. for $C_{27}H_{38}O_6$: C, 70.72; H, 8.35. Found: C, 70.49; H, 8.56.

2*e*. The crude 17β-furyl derivative **2***d* was acetylated for 4 days at room temperature; yield 55% from **1***b*, ethyl acetate – hexane, m.p. 169–171°, $[\alpha]_D$ +51.0°.

Anal. Calcd. for $C_{49}H_{72}O_{16}$: C, 64.18; H, 7.91. Found: C, 63.89; H, 7.89.

17β-[3-Furyl]-3β-tetrahydropyranyloxy-5,14-dihydroxy-5β,14β-androstan-19-al (17)

A mixture of the 19-alcohol (**16***a*) (3.4 g) in acetonitrile (680 ml) and activated neutral MnO_2 (68 g) was stirred overnight at room temperature. It was then filtrated and the filtrate evaporated. The residue obtained was used as such for the following reaction.

The n.m.r. ($CDCl_3$): 0.85 (C-18, CH_3), 4.71 (3-H, M), 6.4 (C-4, H-furyl) 7.17 (C-5, H-furyl), 7.24 (C-2, H-furyl), and 10.2 p.p.m. (CHO, S).

17β-[3-Furyl]-3β,5,14-trihydroxy-5β,14β-androstan-19-al 3-Chloracetate (18)

A solution of the 3-THP ether (**17**) (2.8 g) in ethanol (90 ml) and 0.1 *N* hydrochloric acid (30 ml) was stirred for 4 h at 45–50°. The mixture was then concentrated *in vacuo* to half its original volume and poured into ice cold water. The mixture was extracted with ethyl acetate and the organic solution washed with salt saturated water, dried, and evaporated to give the 3-alcohol (2.3 g).

That alcohol (1.8 g) in pyridine (1.3 ml) and dioxane (13 ml) at 10° was reacted with chloroacetyl chloride (1 ml) in dioxane (3 ml). The addition of the acid chloride was done over a period of 5 min. The mixture was then stirred at the same temperature for 1 h and neutralized with 10% ammonium hydroxide. It was diluted with water and extracted with ethyl acetate. The organic solution was washed with salt saturated water, dried, and evaporated. The crude product (2.0 g) was chromatographed on silica gel (benzene – ethyl acetate 3:1) to afford the title product (1.5 g).

The analytical sample crystallized twice from ethyl acetate – hexane, m.p. 211–213°, $[\alpha]_D$ +8.8°.

Anal. Calcd. for $C_{25}H_{33}ClO_6$: C, 64.59; H, 7.15. Found: C, 64.85; H, 7.16.

The i.r. ν_{max}($CHCl_3$): 3580, 1730, 1705, 1015, and 865 cm^{-1}; n.m.r. ($CDCl_3$): 0.8 (C-18, CH_3), 4.06 (O, $ClCH_2C$—O, 2H), 6.4 (C-4, H-furyl), 7.17 (C-5, H-furyl), 7.24 (C-2, H-furyl), and 10.2 p.p.m. (CHO, S).

In a similar way chloroacetylation of **2***a* and **2***f* gave, respectively:

2*c*. Yield 70% (from **1***a*), ether–hexane, m.p. 166–168°. The n.m.r. ($CDCl_3$): 4.04 p.p.m. ($ClCH_2C$—). After hydrolysis with sodium bicarbonate and acetylation, the product obtained was identical with **2***b*.

2*h*. Yield 70% (from **1***c*), benzene–ether–hexane, m.p. 162–164°, $[\alpha]_D$ +24.7°.

Anal. Calcd. for $C_{27}H_{36}Cl_2O_6$: C, 61.47; H, 6.87; Cl, 13.44. Found: C, 61.34; H, 6.92; Cl, 13.45.

21,23-Dinor-5β,14β-chol-20-ene-3β,14,20,24-tetrol (8a)

To a stirred solution at −20°, of digitoxigenin (500 mg) in dry THF (10 ml) was added dropwise, under nitrogen, a solution of diisobutylaluminum hydride (30% in hexane, 8.6 ml, diluted with dry THF (8.6 ml)). The solution was then stirred at room temperature for 1½ h. A saturated solution of Rochelle salt was then added and the solid obtained removed by filtration and washed carefully with chloroform–EtOH, 95:5. The filtrate was washed with salt saturated water, dried, and evaporated to give the crude title product **8***a*. It was identified as the corresponding triacetate **8***b*. That product was crystallized from CH_2Cl_2–ether, m.p. 120–122°.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.75. Found: C, 69.11, H, 9.10.

The i.r. ν_{max}($CHCl_3$): 3600, 1732, 1220, and 1018 cm^{-1}; n.m.r. ($CDCl_3$): 0.8 (C-18, CH_3), 0.95 (C-19, CH_3), 2.05 (acetates), 4.70 (C-24, CH_2, J = 6 Hz, doublet), 4.75 (C-20, CH_2), and 5.75 p.p.m. (C-22 H, J = 6 Hz, triplet).

17β-[3-Furyl]-5β,14β-androstane-3β,14-diol 3-Acetate (2b)

To a stirred solution at 15° of the crude tetrol (**8***a*) (5.0 g) in DMSO (14 ml) and triethylamine (16 ml) was added dropwise a solution of triethylamine – sulfur trioxide complex (5.0 g) in DMSO (15 ml). After 15 min, the reaction was poured into water (200 ml) and the amine neutralized with 10% sulfuric acid. The mixture was extracted with $CHCl_3$ and the organic layer washed with water, dried, and evaporated. The crude product obtained was acetylated and worked out the usual way. It was then crystallized from methanol to give the title product (3.5 g) as proven by mixture melting point and i.r. analysis.

17β-Isomeric Lactones (3, 19)

3β,14,23-Trihydroxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone 3-Acetate (3a)

Procedure A. To a solution of the furyl derivative **2***b* (2.00 g) in dioxane (100 ml) and water (8 ml) was added portionwise *N*-bromosuccinimide (0.90 g) over a period of 5 min. The solution was then stirred for 30 min and diluted with ether. The ethereal fraction was washed with salt saturated water, dried, and evaporated. The oil obtained was dissolved in acetic acid (100 ml) and stirred in the presence of zinc dust (10 g) for ½ h. The zinc was removed by filtration and the filtrate diluted with chloroform. The organic solution was washed with water until neutral, dried, and evaporated. The product obtained was chromatographed on silica gel; the title product was crystallized from methylene chloride – ether, m.p. 172–173°, $[\alpha]_D$ −5.6°.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.71; H, 8.46.

The i.r. ν_{max}($CHCl_3$): 3620, 3440, 1753, 1737 (inflexion), and 1725 cm^{-1}; n.m.r. ($CDCl_3$): 0.80 (C-18, CH_3), 1.0 (C-19, CH_3), 2.07 (C-3, OAc), 4.83 (C-23, CH_2), and 7.37 p.p.m. (C-22, CH).

Procedure B. A solution of *N,N*-dimethyldichlorohydantoin (181 mg) in dioxane (6 ml) was added dropwise, over a period of 10 min, to a stirred solution at 50° of **2***b* (0.750 g) in dioxane (14 ml), acetic acid (2 ml), and water (2 ml). The solution was stirred at the mentioned temperature for 5 more min and diluted with ether. The product was isolated, treated with zinc in acetic acid, and purified as in procedure A. The pure product was identical with **3***a* as shown by mixture melting point and i.r. analysis, yield, 30%.

The following products were made according to procedure A. **3***k*. Yield 25%, methylene chloride – ether, m.p. 224–228°, $[\alpha]_D$ +25.7°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 67.97; H, 7.82.

3*p*. Yield 25%, methylene chloride – ether, m.p. 228–230°, $[\alpha]_D$ +6.3°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.03, H, 8.06.

The following products were made according to procedure B. **3***i*. Yield 45%, ether–hexane, m.p. 139–142°, $[\alpha]_D$ +45.7°. In this case 1.7 equiv. of *N,N*-dimethylhydantoin had to be used for optimum yield.

Anal. Calcd. for $C_{49}H_{72}O_{17}$: C, 63.07; H, 7.78. Found: C, 62.77; H, 7.91.

3*b*. Yield 55%, methylene chloride – ether–hexane, m.p. 199–201°. The n.m.r. ($CDCl_3$): 4.07 p.p.m. ($ClCH_2C$—). **3***l*. Yield 50%, benzene–ether–hexane, m.p. 129–131°. The n.m.r. ($CDCl_3$): 4.07 and 4.18 p.p.m. ($ClCH_2C$—). **19***a*. Yield 30%. That product, after chromatography, could not be crystallized and was used as such to get **19***b*.

Hydrolysis of Esters

14,23-Dihydroxy-3β-tridigitoxosyloxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3j)

A solution of the lactone **2***i* (3.0 g) and sodium bicarbonate (3.14 g) in methanol (350 ml) and water (90 ml) was kept at 37° for 14 days. The methanol was then evaporated and the aqueous solution extracted three times with $CHCl_3$–EtOH, 2:1. The organic solution was washed with salt saturated water, dried, and evaporated. The crude product was chromatographed (ethyl acetate – acetone, 75:25) to yield the title compound. Crystallization from $CHCl_3$–ether – petroleum ether (30–65°) gave the analytical sample (0.735 g), m.p. 185–190°, $[\alpha]_D$ −8.5°.

Anal. Calcd. for $C_{41}H_{64}O_3$: C, 63.47; H, 8.43. Found: C, 63.59; H, 8.60.

The n.m.r. ($CDCl_3$): 0.7 (C-18, CH_3), 0.87 (C-19, CH_3), 4.82 (CH_2—O—, lactone), and 7.47 p.p.m. (=CH, lactone).

In a similar way was obtained **3***m*. Yield 55%, acetone – ethyl acetate – hexane, m.p. 234–236°, $[\alpha]_D$ +2.5°.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.67; H, 8.75.

3β,14,23-Trihydroxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3c)

A solution of the 3-chloroacetate **3***b* (14.6 g) and potassium bicarbonate (4.6 g) in methanol (1300 ml) and water (160 ml) was refluxed for 3 h. Most of the methanol was removed under reduced pressure, the mixture diluted with water, and extracted with ether. The ethereal solution was washed with salt-saturated water, dried, and evaporated. The product obtained was chromatographed on silica gel (ethyl acetate – benzene, 1:1) to afford the title product.

After acetylation, that compound was identical with **3***a* as shown by mixture melting point and i.r. analysis.

In a similar fashion was obtained **19***b*. Yield 70%, methanol, m.p. 201–204°, $[\alpha]_D$ +17.9°.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.48; H, 8.01.

The n.m.r. ($CDCl_3$): 0.8 (C-18, CH_3), 3.45, 4.15 (OH), 4.8 (CH_2-O, lactone), 7.25 (=CH, lactone), and 10.02 p.p.m. (CHO, S).

Pyranosyloxy Derivatives

3β-(β-D-Tetraacetylglucopyranosyloxy)-14,23-dihydroxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3d)

A mixture of alcohol **3***c* (9.0 g), dried magnesium sulfate (27 g), acetobromoglucose (20 g) was stirred at room temperature, under nitrogen, protected from light, in dry dichloroethane (150 ml) (free from alcohols). After 15 min, dry freshly prepared silver carbonate (15 g) was added and the mixture stirred for 1 h more.

The silver and magnesium salts were removed by filtration and the filtrate evaporated under vacuum. The crude product was dissolved in a minimum amount of methylene chloride, at room temperature, and precipitated with ether. Crystallization from CH_2Cl_2–ether gave the title product (4.0 g, 1st crop; 2.0 g, 2nd crop).

The analytical sample was crystallized from methylene chloride – ethanol, m.p. 206–209°, $[\alpha]_D^{EtOH}$ −57.4°.

Anal. Calcd. for $C_{37}H_{52}O_{13}$: C, 63.05; H, 7.44. Found: C, 62.87; H, 7.14.

The n.m.r. ($CDCl_3$): 0.8 (C-18, CH_3), 0.9 (C-19, CH_3), 2.0 and 2.08 (O$\overset{O}{\overset{\|}{C}}$—$CH_3$, 12 protons), 2.9 (C-14, OH), 4.8 (CH_2-O, lactone), and 7.28 p.p.m. (=CH, lactone).

Mother liquors were chromatographed on silica gel to afford the orthoester (**3***f*). Crystallization from pentane gave the analytical sample (1.0 g), m.p. 105–107°, $[\alpha]_D$ +24.4°.

Anal. Calcd. for $C_{27}H_{52}O_{13}$: C, 63.05; H, 7.44. Found: C, 63.19; H, 7.42.

The n.m.r. ($CDCl_3$): 0.8 (C-18, CH_3), 0.92 (C-19, CH_3), 1.73 (orthoacetate, CH_3), 2.78 (C-14, OH), 4.82 (CH_2—O, lactone, triplet J = 2.5 Hz), and 7.3 p.p.m. (=CH, lactone, doublet J = 5.5 Hz).

This product in acetone containing a trace of hydrochloric acid gave **3***c*.

3β-(β-D-Glucopyranosyloxy)-14,23-dihydroxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3e)

A solution of the acetylated glucosyl derivative **3***d* (1.0 g) and potassium bicarbonate (470 mg) in methanol (180 ml) and water (20 ml) was stirred at room temperature for 10 days. Methanol was then evaporated at reduced pressure (bath temperature < 30°) until a precipitate appeared. The mixture was diluted with water and extracted with ether, chloroform, chloroform–ethanol (9:1 3 × 100 ml fractions, 9:2 3 × 100 ml fractions). The last three fractions were evaporated (0.600 g) and the product crystallized from methanol–water (450 mg), m.p. 237–240°, $[\alpha]_D^{EtOH}$ −39.4°.

[M] Calcd. for **3***b* + methyl β-D-glucopyranoside: (−2300°) + (−6600°) = −8900°. [M] Calcd. for **3***b* + methyl α-D-glucopyranoside: −2300° + 30 800° = + 28 500°. Found for **3***e*: −21 000°. The glycosidic linkage in **3***e* has the β configuration.

Anal. Calcd. for $C_{29}H_{44}O_9$: C, 64.90; H, 8.23. Found: C, 64.97; H, 8.25.

The n.m.r. ($CDCl_3$): 0.7 (C-18, CH_3), 0.88 (C-19, CH_3), 3.27, 3.78, 4.71 (OH), 4.78 (CH_2—O, lactone), and 7.45 p.p.m. (=CH, lactone).

14,23-Dihydroxy-3β-(α-L-rhamnopyranosyloxy)-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3h)

The coupling of **3***c* (4.0 g) with tribenzoyl-α-L-rhamnopyranosyl bromide was done as for **3***d*. The product isolated was chromatographed on silica gel to give *14,23-dihydroxy-3β-(tribenzoyl-α-L-rhamnopyranosyloxy)-24-nor-5β,14β-chol-20-en-21-oic acid γ-lactone* (**3***g*) (4.0 g).

3*g* was hydrolyzed as for **3***e*. The mixture obtained after evaporation of methanol and dilution with water was extracted with ether, chloroform, chloroform–isopropanol, 4:1 (3 × 200 ml fractions). The last three fractions were dried, evaporated, and crystallized from ethanol–hexane (2.5 g), m.p. 225–228°. The analytical sample, m.p. 228–230° was obtained from the same solvent mixture, $[\alpha]_D^{EtOH}$ −55.4°.

[M] Calcd. for **3***b* + methyl α-L-rhamnoside: (−2300°) + (−11 100°) = −13 400°. [M] Calcd. for **3***b* + methyl β-L-rhamoside: −2300° + 14 600° = +12 300°. Found for **3***h*: −29 000°. The glycosidic linkage in **3***h* has the α configuration.

Anal. Calcd. for $C_{29}H_{44}O_8$: C, 66.90; H, 8.52. Found: C, 66.82; H, 8.47.

The n.m.r. ($CDCl_3$): 0.7 (C-18, CH_3), 0.88 (C-19, CH_3), 1.10 (C-6, rhamosyl CH_3), 3.23, 3.90, 4.37, 4.53 (OH), 4.65 (CH_2—O, lactone), and 7.45 p.p.m. (=CH, lactone).

3β-(β-D-Glucopyranosyloxy)-12β,23-dihydroxy-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (3o)

A mixture of **3***m* (1.7 g) anhydrous magnesium sulfate (4.3 g), and acetobromoglucose (4.0 g) was stirred under nitrogen at 45–50° in dry dichloroethane (100 ml). The flask was protected from light and dry freshly prepared silver carbonate (2.8 g) was added and the whole stirred for 2 h. The product isolated as for **3***d* was chromatographed to give **3***n* (1.7 g).

3*n* was hydrolyzed as for **3***e*. The mixture was extracted

with ether, chloroform, benzene–isopropanol, 9:1, 9:2, and 9:3. The last three fractions were dried, evaporated, and crystallized twice from methanol, (350 mg), m.p. 236–238°, $[\alpha]_D^{EtOH}$ −14.8.

[M] Calcd. for **3***m* + methyl β-D-glucopyranoside: 980–6600° = −5620°. [M] Calcd. for **3***m* + methyl α-D-glucopyranoside: 980 + 30 800° = +31 780°. Found for **3***e*: −8100. The glycosidic linkage in **3***o* has the β-configuration.

Anal. Calcd. for $C_{29}H_{44}O_{10}$: C, 63.03; H, 8.02. Found: C, 63.07; H, 8.03.

The n.m.r. ($CDCl_3$): 0.66 (C-18, CH_3), 0.93 (C-19, CH_3), 4.93 (CH_2—O—, lactone), and 7.60 p.p.m. (=CH, lactone).

*3β-(β-D-Glycopyranosyloxy)-5,14-dihydroxy-19-oxo-24-nor-5β,14β-chol-20-en-21-oic Acid γ-Lactone (**19***d*)

The coupling of **19***b* (0.550 g) with acetobromoglucose was done as for **3***o*. The product obtained was chromatographed on silica gel to give the *tetraacetylated* β-D-*glucopyranosyloxy derivative* **19***c* (320 mg). **19***c* was hydrolyzed as for **3***e*. The mixture was extracted with ether, chloroform, chloroform–isopropanol (9:1, 9:2 (3 × 50 ml fractions), 9:3 (3 × 50 ml fractions)). The last six fractions were dried, evaporated and crystallized from methanol–ether (180 mg), m.p. 233–235°. $[\alpha]_D^{EtOH}$ −11.6°.

[M] Calcd. for **19***b* + methyl β-D-glucopyranoside: 7200 − 6600° = +600°. [M] Calcd. for **19***b* + methyl α-D-glucopyranoside: 7200 + 30 800° = +38 000°. Found for **19***d*: −6200°. The glycoside linkage in **19***d* has the β-configuration.

Anal. Calcd. for $C_{29}H_{42}O_{11}$: C, 61.46; H, 7.46. Found: C, 61.30; H, 7.55.

The n.m.r. (DMSO-*d*): 0.67 (C-18, CH_3), 4.66 (CH_2—O—, lactone), 7.47 (=CH—, lactone), and 10.2 p.p.m. (CHO, S).

*3β,14-Dihydroxy-23-oxo-24-nor-5β,14β-chol-20-en-21-oic Acid δ-Lactone 3-Acetate (**4**)*

To a solution of **3***a* (1.0 g) in dioxane (50 ml) and water (4 ml) was added *N*-bromosuccinimide (0.90 g) and the mixture stirred at room temperature for 5 min. The solution was then diluted with ether and the ethereal solution washed with sodium bicarbonate, salt saturated water, dried, and evaporated. The crude product was crystallized several times from ether (0.350 g), m.p. 220° (dec.). That compound could not be analyzed but was identified from the two products **5** and **6**.

The i.r. ν_{max}(KBr): 1721, 1705, 1675, 1623, and 760 cm^{-1}; n.m.r. ($CDCl_3$): 1.0 (C-18 and 19, $2CH_3$), 6.87 (=CH, 1 proton, J = 7 Hz doublet), and 10.3 p.p.m. (CHO, J = 7 Hz doublet).

*3β,14-Dihydroxy-23-oxo-24-nor-5β,14β-cholan-21-oic Acid δ-Lactone 3-Acetate (**6**)*

A solution of **4** (2.3 g) in acetic acid (230 ml) was stirred with zinc dust (23 g) at room temperature for ½ h. The zinc was removed by filtration and the filtrate diluted with chloroform. The organic solution was washed with water until neutral, dried, and evaporated. The residue was crystallized twice from acetone–hexane, m.p. 179–181°, $[\alpha]_D$ −43.9°.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.14; H, 8.50.

The i.r.: ν_{max}($CHCl_3$): 2840, 2730, and 1720 cm^{-1}; n.m.r. ($CDCl_3$): 9.80 p.p.m. (CHO).

*3β,14-Dihydroxy-24-nor-5β,14β-chol-20-ene-21,23-dioic Acid δ-Lactone 3-Acetate (**5**)*

To a solution of **4** (0.330 g) in ethanol (50 ml) was added silver nitrate (0.217 g) in water (7 ml). While the solution was vigorously stirred, a solution of sodium hydroxide (0.217 g) in water (7 ml) was added dropwise and the mixture stirred at room temperature for 3 h. The silver oxide was then removed, the ethanol evaporated, and the solution poured into ice cold 20% sulfuric acid. The solid obtained was filtered, washed with water, and taken into chloroform. The organic solution was extracted with a 10% sodium carbonate solution and the latter acidified. It was then extracted with chloroform which was washed with water, dried, and evaporated. The residue (0.220 g) was crystallized twice from methylene chloride – ether, m.p. 263–264°, $[\alpha]_D$ −17.5°.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 70.04; H, 8.07.

The i.r. ν_{max}(Nujol): 3450, 1700, and 1640 cm^{-1}; the u.v. (EtOH): λ_{max} 232 nm, ε 13 760.

*3β,14,21ξ-Trihydroxy-5β-card-20(22)-enolide 3,21-Diacetate (**20**)*

(*A*) A mixture of **2***b* (1.0 g), sodium acetate (1.0 g), and peracetic acid (40% solution, 1.0 ml) was stirred at room temperature for 30 min in chloroform. It was then diluted with more chloroform and washed with water until free from peracid, dried, and evaporated. The solid obtained was acetylated overnight. The solution was poured into ice cold water and the mixture extracted with ether. The ethereal solution was washed with diluted sulfuric acid, salt saturated water, dried, and evaporated. The crude product was crystallized twice from ether (0.180 g), m.p. 180–183°, $[\alpha]_D$ −25.9°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.22; H, 7.95.

The n.m.r. ($CDCl_3$): 6.18 (=CH, lactone), and 6.66 p.p.m. (C-21, H).

(*B*) A mixture of **2***b* (0.100 g), sodium acetate (0.1 g), acetic acid (1.0 ml), and *m*-chloroperbenzoic acid (0.125 g) in acetone (10 ml) was stirred at room temperature for 5 h. The solvent was then evaporated to one third its initial volume, poured into water, and the solid obtained filtered, and taken in chloroform. The organic solution was washed with sodium bicarbonate solution, water, dried, and evaporated. The solid obtained was crystallized twice from methylene chloride – ether to give **20** as proven by mixture melting point and i.r. analysis, yield 50%.

*3β,21-Dihydroxy-14,21-oxido-24-nor-5β,14β-chol-20-en-23-oic Acid γ-Lactone 3-Acetate (**21**)*

(*A*) The mother liquors obtained from crystallization of **20** (method *A*) were chromatographed on silica gel to give the title product after crystallization from methylene chloride – ether (0.150 g), m.p. 217–219°, $[\alpha]_D$ +42.9°.

Anal. Calcd. for $C_{25}H_{34}O_5$: C, 72.44; H, 8.27. Found: C, 72.82; H, 8.04.

The n.m.r. ($CDCl_3$): 0.85 (C-18, CH_3), 1.0 (C-19, CH_3), 2.03 (C-3, OAc), 5.66 (=CH, lactone), and 5.73 p.p.m. (C-21, H).

(*B*) A mixture of **2***b* (2.0 g) sodium acetate (2.0 g) and *m*-chloroperbenzoic acid (2.50 g) in acetic acid (100 ml)

was stirred at room temperature for 4 h. It was then diluted with chloroform and washed with sodium bicarbonate and water, dried, and evaporated. The oil obtained was crystallized from methylene chloride – ether (0.685 g), and proven to be identical with **21** by mixture melting point and i.r. analysis.

3β,14-Dihydroxy-5β-card-20-enolide 3-Acetate (Digitoxigenin Acetate) (1b)

A solution of **20** (0.150 g) in methanol (7 ml) and water (1 ml) containing sodium hydroxide (0.014 g) was stirred at 0° in the presence of sodium borohydride (0.150 g), for 1 h. It was then poured into 5% ice cold sulfuric acid. The solid obtained was filtered, washed with water, dried, and crystallized from acetone–ether (0.120 g), m.p. 222–224°, identical with digitoxigenin acetate as proven by mixture melting point and i.r. analysis.

3β,14-Dihydroxy-20-oxo-5β,14β-pregnan-21-oic Acid γ-Lactone 3-Acetate (7)

To a stirred solution of **20** (200 mg) in acetone (10 ml) was added powdered potassium permanganate (200 mg). More permanganate was added (100 and 50 mg) after 10 and 50 min. The mixture was then stirred for 1½ h.

The acetone was evaporated and the solid acidified to p*H* 4 with 5% sulfuric acid and extracted with methylene chloride. The organic solvent was evaporated to a volume of 10 ml and diluted with ether. The mixture was washed with sodium bicarbonate, salt saturated water, dried, and evaporated. The residue was crystallized several times from acetone–hexane, (40 mg), m.p. 235–237°, $[\alpha]_D$ −67.3°; (lit. (7), m.p. 235–238°, $[\alpha]_D$ −66.8°).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.73; H, 8.01.

The same product was obtained under identical conditions from **3a**, **4**, and **21**.

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