with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residual oil was solidified on trituration with ether to give N_1-phthalidyl-N_3-*o*-toluyl-5-fluorouracil (**15**): IR (KBr) 1780, 1740, 1710, 1680, 1660 cm^{-1}; NMR ($CDCl_3$) δ 2.72 (3 H, s, CH_3), 6.70 (1 H, d, J = 6 Hz, C_6H), 7.17-8.33 (9 H, m, Ar H and Ar CH).

Reaction of 5-Fluorouracil with 3-Bromophthalide (Method C). 5-FU (1.30 g, 10 mmol) was reacted with 3-bromophthalide (4.69 g, 22 mmol) in the presence of sodium hydride (0.80 g, 20 mmol) as described for the preparation of **8**. The product was crystallized from $CHCl_3$ to yield N_1-phthalidyl-5-fluorouracil (**6**) as a colorless solid (0.26 g, 10%), which showed identical spectra (IR and NMR) with those of compound **6** prepared by method D. The above $CHCl_3$ filtrate was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$ as an eluent, yielding one of the diastereomers of **12** (**12a**; 0.23 g, 6%): IR (KBr) 1785, 1730, 1690, 1670 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.00 (1 H, d, J = 6 Hz, C_6 H).

Subsequent elution with $CHCl_3$ gave **12**[12] (2.53 g, 64%) and the following elution afforded the other diastereomer of **12** (**12b**; 0.28 g, 7%): IR (KBr) 1770, 1730, 1680 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 6.95 (1 H, d, J = 6 Hz, C_6 H).

Deacylation of *N*-Acyl-*N*-phthalidyl-5-fluorouracils. (a) **Solvolysis in AcOH-EtOH (Method D).** A mixture of **8** (3.00 g), EtOH (450 mL), and AcOH (25 mL) was refluxed for 35 h. After the mixture was cooled, the resulting precipitate was collected and washed with ether to give **6**: IR (KBr) 1790, 1700, 1660 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.43-8.17 (6 H, m, C_6 H, Ar H and Ar CH).

(b) **Hydrolysis in Dilute HCl-EtOH (Method E).** N_1-Acetyl-N_3-phthalidyl-5-fluorouracil (**11**;[11] 4.38 g) was heated with a mixture of 0.05 N HCl (50 mL) and EtOH (50 mL) under reflux, and the solvent was removed. To the residue was added water, and the precipitated crystals were collected and washed with water. The dried crystals were treated with hot $CHCl_3$ (15 mL) for 3 min. The insoluble crystals in $CHCl_3$ were collected to give **10**: IR (KBr) 1780, 1725, 1675 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.38-8.05 (6 H, m, C_6 H, Ar H and Ar CH).

Antitumor Activity against P-388 Leukemia (Table II). Female BDF_1 mice (Charles River, Japan) weighing 17-23 g were used. Five mice for each test group were implanted intraperitoneally with 10^6 cells of P-388.[13] Test solution[14] was administered orally once daily for 9 days, starting 1 day after implantation. The antitumor activity of the compounds was expressed by the ratio of the median survival time of the treated mice (T) to that of the control mice (C).

Antitumor Activity of Compounds 6 and 10 and Tegafur (2) against MH 134 and Meth A Tumors. Five female mice (C3H for MH 134 and BALB/c for Meth A) for each group, weighing 17-23 g, were implanted with one of the tumors: 10^6 cells of MH 134 and 5×10^6 cells of Meth A were implanted subcutaneously in the side of the abdominal region of the mouse. Test solution[14] was administered orally twice daily for 20 days, starting 1 day after implantation. On 21st day, the mice were killed, and the tumor weight was assessed. The antitumor activity of compounds was expressed by the ratio of the median weight of tumors of the treated mice (T) to that of the control mice (C).

Acute Toxicity of Compounds 6 and 10 and Tegafur (2). Ten female ICR mice for each group, weighing 22-30 g, were used. Test solution[14] was administered orally. The LD_{50} value was calculated by the Litchfield and Wilcoxon method[15] from the mortality of mice for 21 days after the administration. In addition, the general behavior of the mice was observed after the administration.

Serum Concentration of 5-FU for Mice Treated with Compounds 6 and 10 and Tegafur (2). Female BDF_1 mice weighing 20-26 g (Charles River, Japan) were subjected to this study. Test solution[14] was administered orally at a dose of 1 mmol/kg (dosage of **6** and **10**, 260 mg/kg). After 0.5, 1, 2, 4, and 7 h, the mice were killed by bleeding under ether anesthesia. The collected blood was centrifuged, and the sera were decanted into tubes. The sera were diluted with saline, acidified, and extracted with $CHCl_3$. In a similar way, 5-FU (aqueous layer) was separated from **6**, **10**, and tegafur (organic layer). 5-FU and tegafur concentrations in the sera were determined biologically by the antibacterial activity against *Staphylococcus aureus* P-209,[16] while the concentrations of compounds **6** and **10** were determined by high-pressure liquid chromatography (Waters Associates, Inc.).

Acknowledgment. We thank President A. Yanagisawa and Director H. O. Takagi, Grelan Pharmaceutical Co., Ltd., for their encouragement. We also thank the Pharmaceutical Institute, Tohoku University, for microanalyses.

(12) A mixture of diastereomers of **12a** and **12b** was formed in a ratio of approximately 1:1, mp 214-217 °C (AcOEt).
(13) R. I. Geran, H. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 8 (1972).
(14) Test solution was prepared by dissolving or suspending the test compound in 0.3% sodium carboxymethylcellulose (CMC).
(15) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99 (1949).
(16) Y. Yasuda, T. Togo, N. Unemi, S. Watanabe, K. Harima, and T. Suzue, *J. Jpn. Soc. Cancer Ther.*, 21, 1711 (1973).

Cardenolide Analogues. 14. Synthesis and Biological Activity of Glucosides of C17β-Modified Derivatives of Digitoxigenin

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An improved method for the synthesis of cardiac glycosides was used to prepare 3β-glucosides of digitoxigenin derivatives in which the 17β side chain was CH=CHX (X = COOH, $CONH_2$, $COCH_3$, CN, or COOR). We compared the inotropic activity of the compounds with that of digitoxigenin glucoside using guinea pig left atria. All compounds were active except for the acid (**7**) and the amide (**8**). The inactivity of the amide, in spite of its favorable shape and high capacity for forming intermolecular hydrogen bonds, is incompatible with some previous structure-activity relationship theories. Of the active genins, glucosidation enhanced activity by a factor of about 2. All glucosides, including those with high potency, showed rapid onset and offset of action. The stepwise fall in potency that occurred when the ester group (CH=CHCOOR) was increased in bulk supported previous suggestions that the portion of the digitalis receptor that interacts with the C17 side chain lies within a cleft.

In 1971 we described[1] a simple route whereby the lactone of digitoxigenin could be replaced with a variety of open-chain moieties to produce compounds of the type shown in Scheme I (**4a-h**). In subsequent publications we de-

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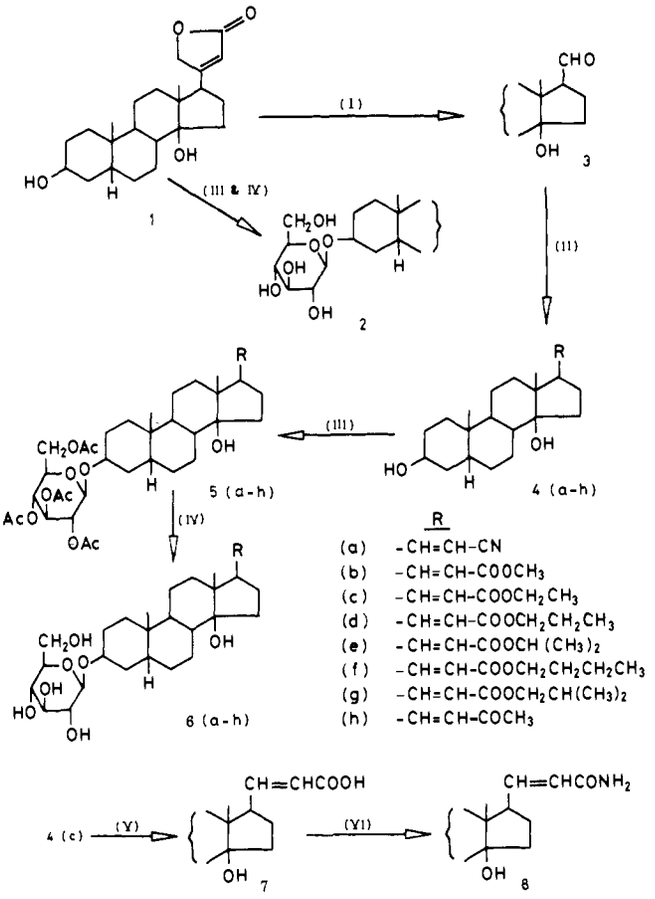
Scheme I. Synthesis of C17β-Modified Derivatives of Digitoxigenin and the Subsequent Conversion of These to Glucosides[a]



[a] I = (a) $(Ac)_2O$/pyridine, (b) O_3, (c) $NaBH_4$, (d) NaOH, (e) $NaIO_4$; II = $(R'O)_2P(O)CH^-R''$; III = Ag_2CO_3 (Celite)/$HgBr_2$/$Hg(CN)_2$; IV = Et_3N/MeOH/H_2O; V = OH^-; VI = Et_3N, $ClCOOCH_2CH(CH_3)_2$, NH_4OH.

scribed the application of this reaction to the study of digitalis SAR.[2] The route has been used by Eberlein and Heider[3] and by Fullerton et al.[4] to prepare C17 analogues of digitoxin and digitoxigenin and by Beard and coworkers[5] to prepare C17 analogues of strophanthidol.

Our earlier work was limited by the fact that certain key compounds had very low water solubility. The compounds included the esters **4b–e** (Scheme I). The low potency and low water solubility of these compounds meant that it was not possible to obtain concentrations high enough to measure the full dose–response curve. To overcome this problem, we synthesized the glucosides of these and related compounds and tested them for inotropic activity using guinea pig left atria. The results of this work are described in this paper.

The advantages of testing glycosides rather than genins are several. Glycosides are usually (but not always) more potent than their corresponding genins. They are also more water soluble. These two sets of properties should enable the full dose–response curves to be obtained and, hence, permit the SAR significance of increasing the size of the C17 side chain to be evaluated.

Chemistry. We prepared compounds as shown in Scheme I using published methods. The etianaldehyde (**3**) was prepared by degradation of the lactone of digitoxigenin.[1] Compounds **4a–h** were prepared by condensing **3** with appropriate phosphonate reagents.[6,7] The glucosides **6a–h** were prepared by reacting **4a–h** with acetobromoglucose in the presence of a mixture of catalysts[8] to give the tetraacetyl glucosides (**5a–h**), which were converted to the free glucosides by mild deacetylation with triethylamine.[8] New compounds described in this report include the glucosides **6a–h** and the aglycons **4f–h** and **8**.

Rigorous proof of structure is essential in all SAR studies, and considerable attention was given to this question in our papers cited above. Chemical and spectroscopic evidence were used to establish that the side chain at C17 was β oriented and that the configuration about the 20,21 double bond was trans.[1,6,7] Proof of structure of glycosides presents a number of problems, since EIMS does not give the molecular ion and ^1H NMR provides limited information about the configuration of the glycosidic linkage. To overcome these problems, we used ammonia CIMS[9] and ^{13}C NMR,[10] together with more traditional methods of structure determination.[8]

Ammonia CIMS of the glucosides gave the molecular ion as the base peak, as well as useful information about purity.[9] With the aid of appropriate reference compounds, it was possible, using ^1H and ^{13}C NMR spectroscopy, to establish that all 3β-glucosides had the β-D configuration.[8,10]

Biological Activity. We tested the compounds for inotropic activity using the isolated left atria of the guinea pig. The concentrations of drug required to increase the force of contraction by 75% (ΔF_{75}) were obtained from the log dose–response curves. Half-times for onset and offset of inotropy were measured after the addition of a single ΔF_{75} dose. These data are shown in Table I, together with corresponding values for a selection of reference compounds. Visual inspection indicated that the dose–response curves were roughly parallel over the initial parts of the curve (including the points at which relative potencies were measured). However, the higher esters did not show the same maximum increase as shown by other active compounds. No reason for this was found, except that it was not due to solubility being exceeded. The importance of the carbohydrate residue in the inhibition of (Na^+,K^+)ATPase by cardiac glycosides was first investigated by Yoda et al.[11] Although these authors examined a number of sugar residues, glucose was not included, nor was the work extended to inotropic activity.

In a previous publication,[8] we drew attention to the range of the contribution that the carbohydrate can make to inotropic activity. For example, we found that digitoxigenin rhamnoside was 22 times more potent than digitoxigenin, digitoxigenin galactoside was equipotent, and digitoxigenin rhamnoside 2′-acetate was half as potent. In the present series, it was not possible to determine the

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Table I. Inotropic Effects on Guinea Pig Left Atria of Genins and Glucosides of C17β-Modified Derivatives of Digitoxigenin

compound	ΔF_{75},[a,d] M·L^{-1}	n, 95% CL[b]	$t_{1/2}$ onset,[c] min (n, ±SE)	$t_{1/2}$ offset,[c] min (n, ±SE)
		Genins		
digitoxigenin (1)	1.1×10^{-6}	12, 75 ± 19	3.0 (6, ±0.2)	
nitrile (4a)	1.6×10^{-6}	12, 75 ± 16	2.1 (3, ±0.3)	
Me ester (4b)	3.8×10^{-6}	9, 75 ± 30	3.1 (4, ±0.7)	
Me ketone (4h)	5.6×10^{-6}	11, 75 ± 24	1.2 (3, ±0.1)	
acid (7)	inactive	4		
amide (8)	<20% at 10^{-4} M	6		
		Glucosides (gluc)		
digitoxigenin gluc (2)	5×10^{-7}	13, 75 ± 16	4.0 (15, ±0.2)	5.7 (12, ±0.4)
nitrile gluc (6a)	6.9×10^{-7}	12, 75 ± 27	3.4 (8, ±0.4)	4.0 (8, ±1.2)
Me ester gluc (6b)	1.5×10^{-6}	22, 75 ± 12	2.6 (15, ±0.2)	5.8 (14, ±0.6)
Et ester gluc (6c)	6.2×10^{-6}	20, 75 ± 9	2.6 (10, ±0.2)	2.9 (10, ±0.2)
n-Pr ester gluc (6d)	1.1×10^{-5} (ΔF_{60})[d]	11, 60 ± 24	2.5 (6, ±0.3)	4.5 (5, ±0.8)
i-Pr ester gluc (6e)	1.5×10^{-5}	9, 75 ± 19	2.6 (10, ±0.3)	4.5 (10, ±0.6)
n-Bu ester gluc (6f)	6.9×10^{-5} (ΔF_{60})[d]	9, 60 ± 14	1.9 (6, ±0.2)	3.2 (4, ±0.8)
i-Bu ester gluc (6g)	6.3×10^{-5}	9, 75 ± 25	2.3 (6, ±0.4)	5.4 (6, ±3.4)
Me ketone gluc (6h)	3.1×10^{-6}	12, 75 ± 23	2.5 (4, ±0.4)	3.0 (3, ±0.6)
		Reference Glycosides		
digoxin	6.6×10^{-7}	11, 75 ± 22	12.2 (10, ±0.7)	18.8 (10, ±1.2)
digitoxin	1.6×10^{-7}	12, 75 ± 22	14.7 (10, ±1.4)	43.1 (10, ±3.7)
ouabain	2.9×10^{-7}	12, 75 ± 19	15.4 (10, ±0.8)	15.8 (10, ±2.0)

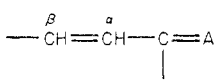
[a] The dose producing a 75% increase in contractility (ΔF_{75}) in guinea pig atria was estimated from the dose-response curves as described under Experimental Section. [b] The number n refers to the number of replicates of the dose-response curve. Confidence limits were interpolated for the ΔF_{75} dose and are given to indicate the spread of observed data in this region. [c] Onset and offset times were determined as described under Experimental Section following the addition of the ΔF_{75} dose as a single dose. The time required for 50% of the effect to develop was called the $t_{1/2}$ onset, and the time required for 50% of the effect to be lost following washout was called the $t_{1/2}$ offset. [d] For compounds 6d and 6f, ΔF_{60} values are given because ΔF_{75} values were not on the linear portion of the dose-response curve.

activity of many of the genins because of lack of solubility. However, for the genins for which full dose–response curves were obtained, namely, for compounds 1 and **4a,b,h**, the addition of the glucose moiety increased activity by factors of, respectively, 2.2, 2.3, 2.5, and 1.8. Thus, in the limited series studied, glucosidation consistently increased activity by a factor of about 2. (The decimals in the relative potencies are not significant.)

Of interest is the fast onset displayed by all glucosides in spite of their 100-fold range in potency.

The stepwise fall in potency that occurred as the C17 side chain was increased in bulk (compounds **6b–g**) confirmed our previous suggestion[2] that the digitalis receptor lies within a cleft that limits the size of the C17 side chain. In our earlier study, the results were obscured by falling solubilities. In the present study, all of the glucosides tested had solubilities in Krebs–Henseleit solution that were greater than the concentrations needed to produce toxic effects. It is possible that a contributing factor to the decreased activity of the higher esters is their increased hydrophobic character. This may inhibit interaction with the receptor or it may lower apparent potency by increasing nonspecific binding, including binding to the walls of the muscle bath.

According to our previous model,[2] the structural feature of the C17 side chain that is essential for digitalis activity is a positive charge. This charge is present in guanylhydrazone analogues[2] and in compounds with α,β-unsaturated side chains of the type:



where A is a hetero atom. Compounds of this type (including those with unsaturated lactones of the cardenolide or bufadienolide type) undergo resonance to produce a fractional positive charge on the β carbon. We have suggested[2] that this fractional charge plays a key role in the drug–receptor interaction and that its binding properties are reinforced by H bonding through the heteroatom. Other authors, in particular Repke[12] and Fullerton,[13,14] have suggested that the carbonyl group (or its heteroatom equivalent) correctly positioned is the prime binding moiety.

It must be emphasized that no one is suggesting that the binding potential of the 17β side chain is sufficient to account for the high affinity that some cardiotonic steroids show for the digitalis receptor. As we and others have stressed, the whole molecule is necessary for optimum effects. Even so, the 17β side chain is essential for activity, possibly because it plays a key role in triggering a major conformational change in the receptor macromolecule that may be essential for biological activity.

The development of suitable probes with which to study the binding potential of the 17β side chain is thus as essential part of digitalis SAR studies. In this context, the amide (8) is of interest because of its *lack* of activity. The amide has the CH=CH–CR=A sequence of atoms that we have postulated as being essential for the isosteric replacement of the lactone. It is also capable of forming powerful H bonds through its carbonyl group. This is of interest because of the suggestion, originally put forward by Repke,[12] that H bonding of this type is the principal way in which the lactone (or its isosteres) interacts with the receptor. Thus, neither the CH=CH—CR=A sequence nor the H-bonding capacity per se are sufficient

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Table II. ^{13}C Chemical Shifts for the β Carbon (C20) of the C17 Side Chains of Digitoxigenin and Related Analogues [a]

compd	chem shift for C20 (β carbon), ppm	rel potency
digitoxigenin (1)	175.3	1
nitrile (4a)	162.6	0.69
Me ester (4b)	155.4	0.29
amide (8)	150.9	<0.01
acid (7) (as K^+ salt)	147.6	inactive
dihydro-digitoxigenin	40.8	<0.05

[a] From Cheung et al.[10]

to account for the contribution that the 17β side chain makes to digitalis activity.

A possible explanation for the lack of activity of the amide is the fact that it lacks the fractional positive charge on the β carbon atom. The lack of resonance-mediated electron withdrawal from the β carbon of the amide was confirmed by ^{13}C NMR spectroscopy (Table II). Alternatively, the conformation of the amide may be inappropriate for effective binding.

This study has confirmed our previous suggestion that the portion of the digitalis receptor that accomodates the C17 side chain of cardiotonic steroids lies within a cleft and that the key binding interaction involves a full or fractional positive charge. We have also shown that glucosidation increases the activity of cardiotonic steroids by a factor of about 2. The presence of a glucose moiety seems to facilitate formation of the drug–receptor complex but detracts from its stability once it is formed.

Experimental Section

Chemistry. Melting points were determined with a Reichert melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer 297 spectrophotometer as 1% dispersions in KCl. ^1H NMR, 100 MHz, spectra were recorded on a Varian HA-100 instrument (tetramethylsilane (δ 0.00) internal standard). Mass spectra were determined on a Finnigan 3200E quadrupole mass spectrometer on line to a Finnigan 6110 data system with ammonia as the reactant gas at a pressure of 1 torr. TLC was performed on plates coated with Silica gel G (Merck), which were developed with chloroform–methanol (9:1). ^{13}C NMR spectra were determined on a Varian CFT-20 spectrometer operating at 20 MHz in the Fourier-transform mode as described by Cheung et al.[10] Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Fêtizon's reagent (silver carbonate finely deposited on Celite) was prepared and used as described by Brown et al.[8]

Preparation of Known Compounds. The etianaldehyde (3), the carboxylic acid (7), and the genins (4a–e) were prepared as described by Boutagy and Thomas[1,6,7] with slight modifications. The glucoside (2) was prepared as described by Brown et al.[8] Phosphonate reagents used for the preparation of 4a–h were prepared as described by Boutagy and Thomas.[15] Acetobromoglucose was prepared as described by Lemieux.[16]

It was found advantageous to reduce the ozonide (first step in the degradation of the lactone) with sodium borohydride. Ammonia CIMS showed that reduction of the ozonide with Adam's catalyst (as used by ourselves and others in previous work) gave a mixture of products, including unreacted ozonide and partially reduced ozonide.

The literature characterization of the phosphonates used in reaction II of Scheme I is generally poor. For this reason, the boiling points of the reagents used are given: diethyl (cyanomethyl)phosphonate, 135 °C (2 mm); dimethyl (methoxycarbonyl)phosphonate, 110–112 °C (3.5 mm); diethyl (ethoxycarbonyl)phosphonate, 133–134 °C (3.5 mm); diethyl [(*n*-propoxycarbonyl)methyl]phosphonate, 142 °C (2 mm); diethyl [(isopropoxycarbonyl)methyl]phosphonate, 116 °C (1.3 mm); diethyl [(*n*-butoxycarbonyl)methyl]phosphonate, 143 °C (2 mm); diethyl [(iso-butoxycarbonyl)methyl]phosphonate, 129–130 °C (1.5 mm); diethyl [(methylcarbonyl)methyl]phosphonate, 108–110 °C (1.2 mm).

Preparation of Genins 4a–h. These compounds were prepared as previously described.[1,6,7] New compounds prepared by this method included **(*E*)-21-(*n*-butoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene** (**4f**) (yield 57% of impure product; mass spectrum, m/z 436 [$(M + NH_4)^+$]), **(*E*)-21-(isobutoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene** (**4g**) (yield 67% of impure product: mass spectrum, m/z 436 [$(M + NH_4)^+$]), and **(*E*)-21-(methylcarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene** (**4h**) (yield 67%; mp 90–92 °C; mass spectrum, m/z 378 [$(M + NH_4)^+$]).

(*E*)-21-Amido-3β,14β-dihydroxy-5β-pregn-20-ene (**8**). (*E*)-3β,14β-Dihydroxy-5β-pregn-20-ene-21-carboxylic acid (7; 0.1 g, 0.3 mmol) was dissolved in dry tetrahydrofuran (3 mL) and treated with triethylamine (3 drops) and isobutyl chloroformate (2 drops). The mixture was left at room temperature for 20 min. Concentrated aqueous ammonia solution (2 mL) was added, and the mixture was stirred in a sealed vessel at room temperature for 2 h. The solution was extracted with chloroform (15 mL), and the chloroform extract was evaporated under reduced pressure to dryness. The residue was dissolved in acetone (3 mL) and water (5 mL) and then evaporated under reduced pressure until the product precipitated as fine, white crystals: yield 69%; mp 138–141 °C; mass spectrum, m/z 362 [$(MH)^+$]. Anal. ($C_{22}H_{35}O_3N$) H, N; C: calcd, 72.67; found, 71.99.

Preparation of Glucosides. Glucosides were prepared as described previously.[8]

(*E*)-21-Cyano-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (**6a**). To a solution of (*E*)-21-cyano-3β,14β-dihydroxy-5β-pregn-20-ene (4a; 0.5 g, 1.5 mmol) in dry benzene (100 mL) was added freshly prepared Fêtizon's reagent (10 g, 17.4 mmol), mercuric cyanide (0.5 g, 1.4 mmol), and mercuric bromide (0.5 g, 2.0 mmol). Acetobromoglucose (4.1 g, 10 mmol) dissolved in dry benzene (20 mL) was added dropwise with stirring over 5 min. The mixture was stirred at room temperature for a further 60 min. It was then filtered through a Celite pad and washed three times with saturated aqueous sodium bicarbonate solution (150 mL). Most of the benzene was removed by evaporation at low temperature. Aqueous potassium bicarbonate solution (1%, 20 mL) was added, and the remaining benzene was removed, resulting in precipitation of the crude product. The residue was dissolved in methanol (50 mL), which was then slowly evaporated until crystallization started. The solution was allowed to stand until crystallization was complete. The product was collected and washed with methanol–water (1:1). Recrystallization from methanol–water gave 0.35 g of product, which consisted mainly of the tetraacetyl derivative (**5a**) plus a small amount of the starting material (4a). The material was deacetylated by allowing it to stand for 72 h at room temperature in a mixture of methanol (20 mL), triethylamine (20 mL), and water (1 mL). The mixture was evaporated to dryness under reduced pressure at room temperature and applied to an alumina column (30 g) as a solution in methanol (3 mL) and chloroform (30 mL). The first column fractions gave unreacted 4a. Elution with chloroform–methanol (4:1) gave pure (*E*)-21-cyano-14β-dihydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (6a; 0.2 g, 0.4 mmol, 28% from 4a): mp 213–215 °C; mass spectrum (NH_3), m/z 523 [$(M + NH_4)^+$]; IR ν_{max} (KCl) 3230, 2930, 2220, 1625, 1450, 1375, 1360, 1270, 1110, 1075, 1025 cm^{-1}; NMR (Polysol-D) δ 0.81 (s, 18-CH_3), 0.91 (s, 19-CH_3), 5.28 (d, $J_{21,20}$ = 15.5 Hz, 20-CH), 7.12 (dd, $J_{20,21}$ = 15.5 Hz, $J_{20,17}$ = 9 Hz, 20-CH). Anal. ($C_{28}H_{43}O_7N \cdot 1H_2O$) H, N; C: calcd, 64.20; found, 64.99.

(*E*)-21-(Methoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (**6b**). (*E*)-21-(Methoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (4b; 0.5 g, 1.3 mmol) was reacted with acetobromoglucose (3.0 g, 7.3 mmol) as described for 6a. Recrystallization from ethanol–water gave pure (*E*)-21-(methoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside tet-

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raacetate (**5b**; 0.55 g, 0.8 mmol, 59%): mp 103–108 °C; mass spectrum (NH_3), m/z 724 [$(M + NH_4)^+$].

Deacetylation of **5b** (0.4 g, 0.57 mmol) as described for **5a** gave pure (*E*)-21-(methoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6b**; 0.3 g, 0.57 mmol, 58% from **4b**): mp 138–142 °C; mass spectrum (NH_3), m/z 556 [$(M + NH_4)^+$]; IR ν_{max} (KCl) 3400, 2940, 1700, 1640, 1450, 1360, 1260, 1220, 1165, 1075, 1020 cm^{-1}; NMR (15% Me_2SO-d_6 in $CDCl_3$) δ 0.86 (s, 18-CH_3), 0.94 (s, 19-CH_3), 3.70 (s, OCH_3) 4.36 (d, $J_{1',2'}$ = 7.5 Hz, 1'-CH), 5.6 (d, $J_{21,20}$ = 17 Hz, 21-CH), 7.24 (dd, $J_{20,21}$ = 17 Hz, $J_{20,17}$ = 9 Hz, 20-CH). Anal. ($C_{29}H_{46}O_9 \cdot 1H_2O$) C, H.

(*E*)-21-(Ethoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (6c). (*E*)-21-(Ethoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4c**; 0.3 g, 0.8 mmol) was reacted with acetobromoglucose (3.0 g, 7.3 mmol) as described for **6a**. Recrystallization from ethanol–water gave pure (*E*)-21-(ethoxycarbonyl)-14β-hydroxypregn-20-en-3β-yl β-D-glucoside tetraacetate (**5c**; 0.35 g, 0.49 mmol, 63%): mp 164–165 °C; mass spectrum (NH_3), m/z 738 [$(M + NH_4)^+$].

Deacetylation of **5c** (0.35 g, 0.49 mmol) as described for **5a** gave, after recrystallization from ethanol–water, pure (*E*)-21-(ethoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6c**; 0.26 g, 0.47 mmol, 61% from **4c**): mp 128–131 °C; mass spectrum (NH_3), m/z 570 [$(M + NH_4)^+$]; IR ν_{max} (KCl) 3360, 2920, 1695, 1640, 1445, 1365, 1255, 1220, 1155, 1070, 1015 cm^{-1}; NMR (15% Me_2SO-d_6 in $CDCl_3$) δ 0.86 (s, 18-CH_3), 0.95 (s, 19-CH_3), 4.12 (d, $J_{1',2'}$ = 7.5 Hz, 1'-CH), 5.52 (d, $J_{21,20}$ = 16 Hz, 21-CH), 7.20 (dd, $J_{20,21}$ = 16 Hz, $J_{20,17}$ = 9 Hz, 20-CH). Anal. ($C_{30}H_{48}O_9 \cdot 1H_2O$) C, H.

(*E*)-21-(*n*-Propoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (6d). (*E*)-21-(*n*-Propoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4d**; 0.3 g, 0.74 mmol) was reacted with acetobromoglucose (3.0 g, 7.3 mmol) as described for **6a**. Recrystallization from ethanol–water gave pure (*E*)-21-(*n*-propoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside tetraacetate (**5d**; 0.35 g, 0.48 mmol, 65%): mp 155–156 °C; mass spectrum (NH_3), m/z 752 [$(M + NH_4)^+$].

Deacetylation as described for **5a** gave, after recrystallization from ethanol–water, pure (*E*)-21-(*n*-propoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6d**; 0.19 g, 0.34 mmol, 45% from **4d**), mp 116–119, 154–155 °C; mass spectrum (NH_3), m/z 584 [$(M + NH_4)^+$]; IR ν_{max} (KCl) 3340, 2920, 1700, 1640, 1445, 1375, 1350, 1260, 1215, 1160, 1075, 1020 cm^{-1}; NMR (15% Me_2SO-d_6 in $CDCl_3$) δ 0.84 (s, 18-CH_3), 0.92 (overlapping s and t; s, 19-CH_3; t, J = 7 Hz, $COOCH_2CH_2CH_3$), 3.98 (t, J = 6 Hz, $COOCH_2CH_2CH_3$), 4.35 (d, $J_{1',2'}$ = 6 Hz, 1'-CH), 5.60 (d, $J_{21,20}$ = 16 Hz, 21-CH), 7.24 (dd, $J_{20,21}$ = 16 Hz, $J_{20,17}$ = 9.5 Hz, 20-CH). Anal. ($C_{31}H_{50}O_9 \cdot 1H_2O$) C, H.

(*E*)-21-(Isopropoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (6e). (*E*)-21-(Isopropoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4e**; 0.4 g, 1 mmol) was reacted with acetobromoglucose (3.0 g, 7.3 mmol) as described for **6a**. TLC showed that much unreacted **4e** was present with the product. No attempt was made to obtain a pure sample of **5e**. Deacetylation was performed on this mixture, and column chromatography was used for separation. The first fractions gave unreacted starting material; elution with chloroform–methanol (4:1) gave pure (*E*)-21-(isopropoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6e**; 0.1 g, 0.18 mmol, 18% from **4e**): mp 138–142 °C; mass spectrum (NH_3), m/z 584 [$(M + NH_4)^+$]. Anal. ($C_{31}H_{50}O_9 \cdot 1H_2O$) H, C: calcd, 63.66; found, 62.92.

(*E*)-21-(*n*-Butoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (6f). (*E*)-21-(*n*-Butoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4f**; 0.6 g, 1.44 mmol) was reacted with acetobromoglucose (6.0 g, 14.6 mmol) as described for **6a**. TLC showed the presence of some unreacted starting material (**4f**); recrystallization of a portion gave pure (*E*)-21-(*n*-butoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside tetraacetate (**5f**; 0.1 g, 0.13 mmol): mp 85–88 °C; mass spectrum (NH_3), m/z 766 [$(M + NH_4)^+$].

Deacetylation as described for **5a** gave pure (*E*)-21-(*n*-butoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6f**; 0.24 g, 0.41 mmol, 29% from **4f**): mp 113–118 °C; mass spectrum (NH_3), m/z 598 [$(M + NH_4)^+$]. Anal. ($C_{32}H_{52}O_9 \cdot 1.5H_2O$) C, H.

(*E*)-21-(Isobutoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (6g). (*E*)-21-(Isobutoxycarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4g**; 0.5 g, 1.2 mmol) was reacted with acetobromoglucose (4.1 g, 10 mmol) as described for **6a**. TLC showed the presence of unreacted starting material (**4g**) in addition to the β-D-glucoside tetraacetate **5g**. A small portion was recrystallized several times from ethanol–water to give pure (*E*)-21-(isobutoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside tetraacetate (**5g**; 0.002 g), mass spectrum (NH_3), m/z 766 [$(M + NH_4)^+$], essentially free of **4g**, m/z 436 [$(M + NH_4)^+$].

Deacetylation of the glycosidation reaction products, followed by column chromatography and recrystallization from ethanol–water, gave pure (*E*)-21-(isobutoxycarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6g**; 0.16 g, 0.27 mmol, 23% from **4g**), mp 120–122/160–162 °C; mass spectrum (NH_3), m/z 598 [$(M + NH_4)^+$]. Anal. ($C_{32}H_{52}O_9 \cdot 1.5H_2O$) C, H.

(*E*)-21-(Methylcarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-Glucoside (4h). (*E*)-21-(Methylcarbonyl)-3β,14β-dihydroxy-5β-pregn-20-ene (**4h**; 0.4 g, 1.1 mmol) was reacted with acetobromoglucose (4.1 g, 10 mmol) as described for **6a**. A portion of the glycosidation product was recrystallized from ethanol–water to remove a small amount of unreacted starting material (**4h**) to give pure (*E*)-21-(methylcarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside tetraacetate (**5h**; 0.05 g, 0.07 mmol): mp 141–144/175–184 °C; mass spectrum (NH_3), m/z 708 [$(M + NH_4)^+$].

Deacetylation as described for **5a** gave pure (*E*)-21-(methylcarbonyl)-14β-hydroxy-5β-pregn-20-en-3β-yl β-D-glucoside (**6h**; 0.18 g, 0.34 mmol, 31% from **4h**): mp 192–194/232–235 °C; mass spectrum (NH_3), m/z 540 [$(M + NH_4)^+$]. Anal. ($C_{29}H_{40}O_8$) C, H.

Biological Methods. We determined inotropic activity using guinea pig left atria suspended in Krebs–Henseleit solution (gassed with 95% O_2/5% CO_2) at 32 °C. Resting tension was adjusted to 1 g after 1 h of equilibration. The atria were stimulated by rectangular pulses (10-ms duration) at a frequency of 90/min. The voltage was set at 20% above threshold, minimum 5 V. Cumulative dose–response curves were obtained by adding to the bath aliquots of drug in propylene glycol/water (7:3). Aliquots were added at 30-min intervals or, if this period was too short, at 5 min after maximum effect had been reached. We calculated changes in isometric contraction with reference to changes in control atria using the protocol described previously.[17]

We measured at least nine replicates of each dose–response curve and obtained the curve of best fit using the SPSS program (Vogelback Computing Center, Northwestern University). The dose producing a 75% increase in force (ΔF_{75}) was read from the fitted curve for pooled data.

Onset and offset times were obtained by adding the ΔF_{75} dose (obtained from the dose–response curve) as a single dose. In this way it was hoped to compare equieffective doses. Fifteen minutes after maximum effect was achieved, the tissue was washed quickly with three changes of drug-free medium and then allowed to equilibrate. Since it is not possible to determine the precise moment that equilibrium is established, following the addition and washout of a dose, the time required for the first 50% of effect (onset or washout) was measured. These values were called the $t_{1/2}$ onset and $t_{1/2}$ offset values and are given in Table I.

We determined the maximum solubilities of compounds **6d–g** in Krebs–Henseleit medium by shaking excess solid drug in the medium at 32 °C for 16 h. The suspensions were then filtered, and the concentration of drug in the filtrate was determined by UV spectroscopy. By this means is was established that the maximum test dose used was within its range of solubility.

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