solid. Recrystallization from acetonitrile afforded **14q**, 2.49 g (76%), mp 134–6 °C. Anal. ($C_{10}H_{13}FN_2O_2$) C, H, N. NMR ($CDCl_3$) δ 3.28 (4 H, m, 2 × CH_2N), 3.85 (4 H, m, 2 × CH_2O), 4.65 (2 H, s, CH_2OH), 6.73 (1 H, d, J = 7 Hz, py-3H), 8.16 (1 H, d, J = 5 Hz, py-6H).

2-[[(5-Fluoro-4-morpholino-2-pyridyl)methyl]sulfinyl]-5-methoxy-(1*H*)-benzimidazole (3q). Compound **14q** was similarly converted to **3q** via the corresponding 2-(chloromethyl)-pyridine **4q** and sulfide **6q** by analogy with method A, overall yield 59%, mp 109–11 °C. Anal. ($C_{18}H_{19}FN_4O_3S·0.15H_2O$) C, H, N, S. NMR (DMSO-$d_6$) δ 2.95 (4 H, m, 2 × CH_2N), 3.61 (4 H, m, 2 × CH_2O), 3.80 (3 H, s, OCH_3), 4.58 (2 H, d of d, J = 13 Hz, CH_2SO), 6.59 (1 H, d, J = 8 Hz, py-3H), 6.92 (1 H, d of d, J = 9 Hz, 2.5 Hz, benzim-6H), 7.08 (1 H, d, J = 2.5 Hz, benzim-4H), 7.54 (1 H, d, J = 9 Hz, benzim-7H), 8.20 (1 H, d, J = 6 Hz, py-6H); IR (Nujol) ν_{max} 3400–2450 (NH), 1600, 1590 (C=N + ring modes), 1100, 1060 (S=O and C–O) cm^{-1}.

Stability and pK_a Measurements. Stability at ambient temperature (20 °C) was determined in acetonitrile/phosphate buffer (1:3) which had been adjusted to the appropriate pH. The initial concentration of compounds was between 1 × 10^{-3} and 3 × 10^{-4} M, and the decrease in concentration was monitored by HPLC (μBondapak C_{18}, CH_3CN/KH_2PO_4, pH 7.4, 20–80% CH_3CN in 25 min, 1 mL min^{-1}, 40 °C, detector UV 280 nm). Half-lives ($t_{1/2}$) were determined from the linear regression of ln of the concentration vs time (h).

The pK_a's of the substituted 2-(hydroxymethyl)pyridines were measured spectrophotometrically at 37 °C.

In Vivo Biological Assays. Initial studies were carried out by using the stomach lumen perfusion technique in the anaesthetized rat with continuous monitoring of the H^+ activity of the effluent perfusate using a microflow glass electrode. Acid secretion was stimulated by an intravenous infusion of histamine at 0.25 μmol/(kg·min), and the inhibitors were given either intravenously or intraduodenally when a submaximal plateau of secretion had been achieved. Percentage inhibition was calculated from a comparison of the acid secretion at peak inhibition compared to the secretory plateau immediately prior to administration. ED_{50} values were obtained by using three dose levels with three animals at each dose level.

Studies were also carried out in the conscious dog surgically prepared with a Heidenhain pouch 1–3 years previously. Acid secretion was stimulated by an intravenous infusion of histamine at 0.5 μmol/(kg·h) and gastric juice collected by gravity drainage at 15-min intervals. An aliquot of the juice was titrated to pH 7.4 with 0.1 N sodium hydroxide and the acid output calculated from the product of the acid concentration and volume of gastric juice secreted. Inhibitors were given either intravenously or intraduodenally, through a chronically implanted duodenal cannula, when a submaximal plateau of secretion had been established. Percentage inhibition was calculated from a comparison of the acid output in the 15-min sample at peak inhibition with the acid output in the sample immediately prior to compound administration. ED_{50} values were calculated by using three dose levels with three animals at each dose level.

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Cardiac Glycoside-like Structure and Function of 5β,14β-Pregnanes

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5β-Reduction and 14β-substitution convert the planar progesterone molecule to the cardiac glycoside configuration—A and D rings of the steroid moiety are bent toward the α-face relative to the B and C rings. Potency of the 5β,14β-derivative in a [^{3}H]ouabain binding assay or its ability to inhibit the sodium pump in red blood cells is enhanced by 3β-hydroxylation, 20β-hydroxylation, and 3β-glycosidation. Synthesis of 14,20β-dihydroxy-3β-(β-D-glucopyranosyloxy)-5β,14β-pregnane from digitoxin is described. The glucoside is $^1/_{20}$ as potent as ouabain and elicits prominent, sustained, positive inotropy in isolated cardiac muscle.

The high-affinity binding of the cardiac glycosides to its biological receptor, i.e. Na^+,K^+-ATPase, is noted also for its high degree of structural specificity.[1] In previous studies from our laboratory,[2-6] it was demonstrated that certain derivatives of progesterone are inhibitors of [^{3}H]ouabain binding to cell-membrane preparations and inhibit the sodium pump in isolated tissues. The most active congeners previously identified are represented by chlormadinone acetate (17α-acetoxy-6-chloropregna-4,6-diene-3,20-dione; CMA). CMA and related compounds, however, in contrast to the cardiostimulant cardiac glycosides, produce only transient positive inotropy associated with progressively developing cardiodepression. Important structural distinctions between CMA and the cardiac glycosides may account for this major important difference in myocardial response. For example, whereas the steroid nucleus of the mammalian hormones is essentially planar, in the cardiac glycosides the cis configuration of the A/B and C/D ring junctions orientate the A and D rings almost perpendicular to the B and C rings. We proposed that the apparent paradoxical cardiodepression occurring in association with the binding to and inhibition of Na^+,K^+-ATPase by CMA reflects an additional action, i.e. inhibition by the steroid of a metabolic process. The latter effect may account for depression of myocardial cell function which counteracts positive inotropy resulting from inhibition of the enzyme.

We have pointed out that 5β-reduction and 14β-substitution of the planar progesterone molecule convert the

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(1) Guntert, T. W.; Linde, H. H.A. In *Cardiac Glycosides*; Freef, K., Ed.; Springer-Verlag: Berlin, 1981; Part 1, Chapter 2, pp 13–24.
(2) Chow, E.; Kim, R. S.; LaBella, F. S.; Queen, G. *Br. J. Pharmacol.* **1979**, *67*, 345.
(3) Kim, R. S.; LaBella, F. S.; Zunza, H.; Zunza, F.; Templeton, J. *Mol. Pharmacol.* **1980**, *18*, 402.
(4) LaBella, F. S.; Bihler, I.; Kim, R. S. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1057.
(5) LaBella, F. S.; Bihler, I.; Templeton, J.; Kim, R. S.; Hnatowich, M.; Rohrer, D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 2806.
(6) LaBella, F. S.; Bihler, I.; Kim, R. S. *Nature (London)* **1979**, *28*, 571.

0022-2623/89/1832-1977$01.50/0

Table I. ^{13}C NMR Spectral Data of 5β,14β-Pregnane Derivatives[a]

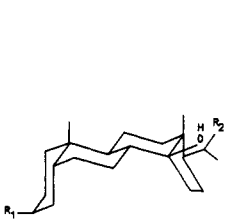
C	4[b]	5[c]	9[c]
1	30.0	(30.4)	30.4
2	(26.3)	[27.2]	28.2
3	74.2	74.2	67.3
4	29.6	(30.6)	33.8
5	36.5	36.8	36.8
6	(26.8)	[27.0]	(27.0)
7	21.4	21.9	22.0
8	40.8	40.8	40.9
9	35.7	36.2	36.1
10	35.2	35.6	36.0
11	20.6	21.0	21.1
12	41.7	41.9	42.0
13	47.7	-	-
14	85.5	85.9	86.1
15	32.2	32.2	32.5
16	(26.5)	[26.7]	(27.4)
17	56.7	56.9	56.9
18	16.3	16.6	16.9
19	23.3	23.2	[23.3]
20	[71.9]	71.8	72.2
21	23.9	23.9	[23.6]
C1′	98.6	101.8	
C2′	[71.7]	75.1	
C3′	72.9	77.2	
C4′	68.7	70.9	
C5′	[71.5]	76.6	
C6′	62.2	62.3	
	169.0[d]		
	169.4[d]		
	170.3[d]		
	170.6[d]		
	20.4 × 4[d]		

[a] Chemical shifts are in ppm (Me_4Si internal standard); enclosed numbers in a column are interchangeable. [b] $CDCl_3$. [c] $CDCl_3$/CD_3OD (1:1). [d] $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{6'}$ acetyl.

A/B and C/D junctions to the cis configuration characteristic of the cardiac glycosides, although this molecular configuration is not essential for eliciting positive inotropy. In the present report, we present our latest findings on the structure–activity relationships of pregnanes that interact at the cardiac glycoside binding site, specifically, the effects of substitutions and glycosidation on the biological properties of 5β,14β derivatives.

Results and Discussion

3β-Acetoxy-14-hydroxy-5β,14β-pregnan-20-one (**1**) was prepared from digitoxin as previously reported[7-11] (see Scheme I). Reduction of 20-ketone **1** with lithium tri-*tert*-butoxyaluminohydride (LTBA) gave 20β-alcohol **2**, which was protected as the (*tert*-butyldimethylsilyl)oxy derivative[12] and reduced with lithium aluminohydride (LAH) to give 3β-alcohol **3**. This alcohol on treatment with tetraacetylbromoglucose,[13] removal of the silyl group[12] to give **4**, and hydrolysis[13] yielded glucoside **5**. Attempts to hydrolyze 3β-acetate **1** with potassium hydrogen carbonate in methanol/water at reflux led mainly to 17α,3β-alcohol **6**[14] and a lesser amount of 3β-alcohol **7**; oxidation of **7** gave dione **8**. LAH reduction of **2** gave triol **9**. Structures were established by ^1H NMR and ^{13}C NMR spectra analyses.



Compound Number	R_1	R_2	IC_{50} ^3H-ouabain binding (uM)
(1)	OAc	=O	20
(2)	OAc	OH	6
(5)[a]	glucose	OH	0.4
(6) (17α)	=O	=O	>400
(7)	OH	=O	21
(8)	=O	=O	46
(9)	OH	OH	6

[a] The corresponding non-crystalline 3β-α-L-rhamnosyl gave an IC_{50} of 0.06.

Figure 1. Structures of 5β,14β-pregnanes and their potencies in the [^3H]ouabain-binding assay.

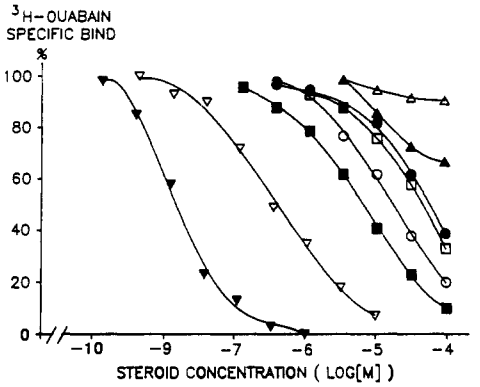


Figure 2. Inhibition of specific binding of [^3H]ouabain to membranes from dog heart: (▼) ouabain, (▽) 14,20β-dihydroxy-3β-(β-D-glucopyranosyloxy)-5β,14β-pregnane (**5**), (■) 3β,14,20β-trihydroxy-5β,14β-pregnane (**9**), (○) 3β,14-dihydroxy-5β,14β-pregnan-20-one (**7**), (□) 14-hydroxy-5β,14β-pregnane-3,20-dione (**8**), (●) progesterone, (▲) pregna-4,14-diene-3,20-dione, (△) 3β,14-dihydroxy-5β,14β,17α-pregnan-20-one (**6**).

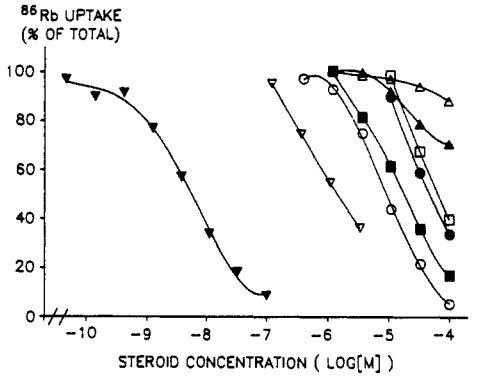


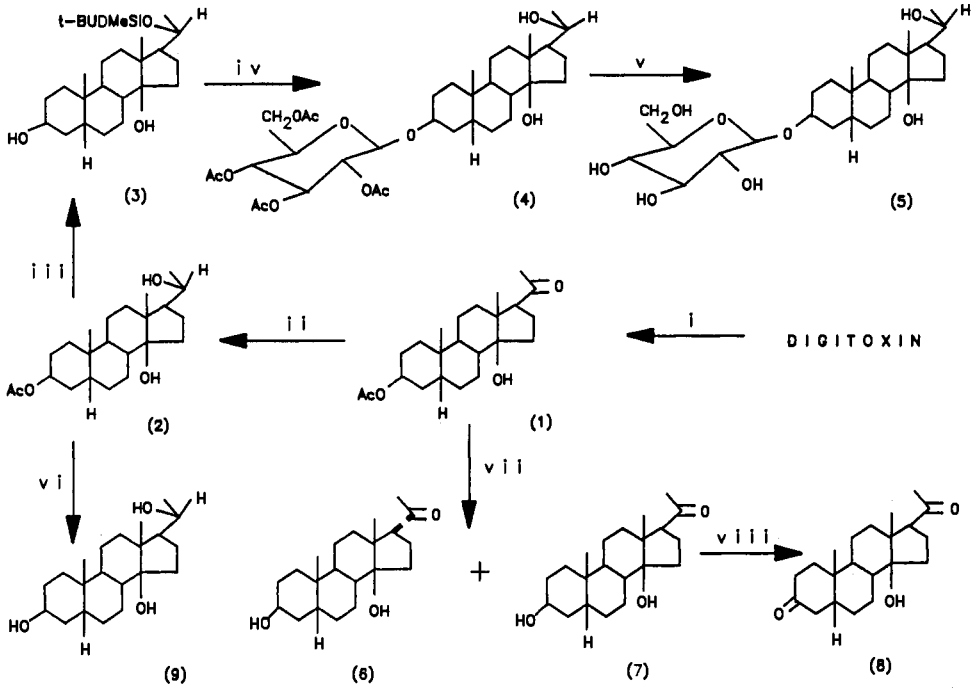
Figure 3. Inhibition by pregnanes of ^{86}Rb uptake by human red blood cells. Symbols are as in Figure 2.

^{13}C NMR assignments (Table I) are based on polarization transfer,[15] internal consistency, and comparison with published data.[16,17]

The 5β,14β-pregnane configuration (A/B junction cis, C/D junction cis) is identical with that of the steroid moiety of the cardiac glycosides; the former has the acetyl and the latter a lactone ring at the 17β-position. We report here a derivative (14,20β-dihydroxy-3β-(β-D-glucopyranosyloxy)-5β,14β-pregnane; glucoside) with $^1/_{20}$ the potency of ouabain (Figure 1). Others have synthesized a derivative (14-amino-3β-(α-L-rhamnopyranosyloxy)-5β,14β-pregnane) equipotent to ouabain.[18,19] Clearly, the

(7) Cloetta, M. *Arch. Exp. Pathol. Pharmakol.* **1920**, *88*, 113.
(8) Jacobs, W. A.; Elderfield, R. C. *J. Biol. Chem.* **1935**, *108*, 497.
(9) Hunzika, F.; Reichstein, T. *Helv. Chem. Acta* **1945**, *28*, 1472.
(10) Baran, J. S. *J. Org. Chem.* **1964**, *29*, 527.
(11) Eppenberger, U.; Vetter, W.; Reichstein, T. *Helv. Chim. Acta* **1966**, *49*, 1505.
(12) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(13) Brown, L.; Boutagy, J.; Thomas, R. *Arzneim.-Forsch./Drug Res.* **1981**, *31(II)*, 1059.
(14) Lindig, C. *J. Prakt, Chem.* **1983**, *4*, 587.
(15) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(16) Habermehl, G. G.; Hamman, P. E.; Wray, V. *Magn. Reson. Chem.* **1985**, *23*, 959.
(17) Robien, W.; Kopp, B.; Schabl, D.; Schwarz, H. *Prog. Nucl. Magn. Reson.* **1987**, *19*, 131.

Scheme I[a]



[a] (i) 0.05 M H_2SO_4/MeOH/H_2O, Ac_2O/py, O_3, Zn/HOAc, *p*-TsCl/py, NaI/acetone, Zn/HOAc; (ii) LTBA; (iii) *t*-$BuMe_2SiCl$/imidazole/DMF, LAH; (iv) acetobromoglucose/Ag_2CO_3/Celite/$Hg(CN)_2$/$HgBr_2$/benzene, *n*-$Bu_4N^+F^-$; (v) Et_3N/MeOH/H_2O; (vi) LAH; (vii) $KHCO_3$; (viii) PDC.

lactone ring is not essential for full activity. Furthermore, we showed that a substituted planar progesterone derivative, CMA, as the aglycon, is $^1/_{15}$ as potent as ouabain.[2-5] CMA lacks the C17-lactone ring, the 14β-OH, and the A/B, C/D cis configurations. (However, in that series, glycosidation results in derivatives with diminished potency.[20])

5α-Pregnane (A/B trans), 5β-pregnane (A/B cis), and 14β-pregnane (C/D cis) all may be substituted to yield active derivatives (unpublished data and ref 21 and 22), indicating that none of the structural features of the digitalis steroids (see ref 1) is absolutely essential for potent biological activity receptor interaction.

The series of 5β,14β-pregnanes give parallel concentration–activity curves in both the [^{3}H]ouabain binding assay (Figure 2) and the ^{86}Rb-uptake assay (Figure 3). In general, the relative potencies of this class of compounds are similar both in the binding assay and in functional assays. One compound (the 3β,14,20β-trihydroxy) shows a slight discrepancy in potencies in the two assays, perhaps reflecting species differences (dog heart membranes and human red cells). Also, Na,K-ATPase isoforms express differential tissue distribution[23] and may vary in their affinities for ligands at the steroid-recognition site.

Glucoside **5** promotes a concentration-dependent increase in contractile force of isolated heart muscle (Figure 4) and the maximum developed tension is equal to that by ouabagenin (Figure 5). Furthermore, the glucoside, like ouabagenin, elicits at high concentration an increase in diastolic tension (Figure 5).

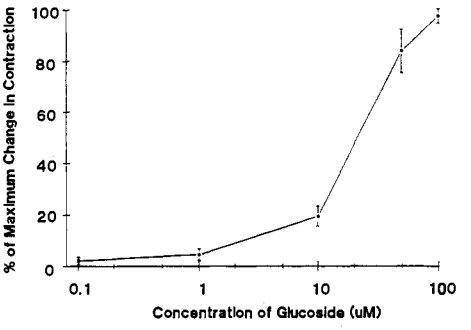


Figure 4. Dose–response curve to glucoside (**5**) on isolated right ventricular trabecula. The effect of the drug on isometric contractile force development is plotted as a function of maximum increase in developed tension. The data are means ± SEM from six experiments.

The positive inotropic effect of the glucoside was decreased very little by 50 μM of the β-adrenoceptor blocker sotalol. However, much larger concentrations (200 and 600 μM) blocked the effect of the glucoside by 10 and 25% (Figure 6) although some of this depression, especially at the highest concentration of the blocker, may be nonspecific. An appreciable component of the positive inotropic response to the glucoside is unrelated to catecholamine release and is presumably a direct action of compound **5**. The present findings support the contention that the binding assay accurately predicts the biological potency of steroids. The planar pregnane CMA[24] and a 5β,14β-androstane,[25] each shown to inhibit [^{3}H]ouabain and

(18) Jarreau, F. X.; Koenig, J. J.; Fenard, S. *Eur. Heart J.* 1984, *5* (suppl. F), 309.
(19) Halpryn, B.; Fulton, R.; Fulton, V.; Panasevich, R.; Ogerau, T.; Fenard, S.; Baggioni, A.; Koenig, J.; Chang, Y. *Pharmacologist* 1987, *29*, 135.
(20) Weiland, J.; Schwabe, K.; Hubler, D.; Chhonfeld, W.; Repke, K. R. H. *J. Enzyme Inhib.* 1987, *2*, 31.
(21) Templeton, J. F.; Sashi Kumar, V. P.; Cote, D.; Bose, D.; Elliott, D.; Kim, R. S.; LaBella, F. S. *J. Med. Chem.* 1987, *30*, 1502.
(22) Templeton, J. F.; Kumar, S. V. P.; Bose, D.; Smyth, D. D.; Kim, R. S.; LaBella, F. S. *Can. J. Physiol. Pharmacol.* 1988, *66*, 1420.
(23) Rossier, B. C.; Geering, K.; Kraehenbuhl, J. P. *Trends Biochem. Sci.* 1987, *12*, 483.
(24) Wehling, M.; Schwartz, A.; Whitmer, K.; Grupp, G.; Grupp, I. L.; Wallick, E. T. *Mol. Pharmacol.* 1981, *20*, 551.
(25) Schonfeld, W.; Menke, K. H.; Schonfeld, R.; Repke, K. R. H. *J. Enzyme Inhib.* 1987, *2*, 37.

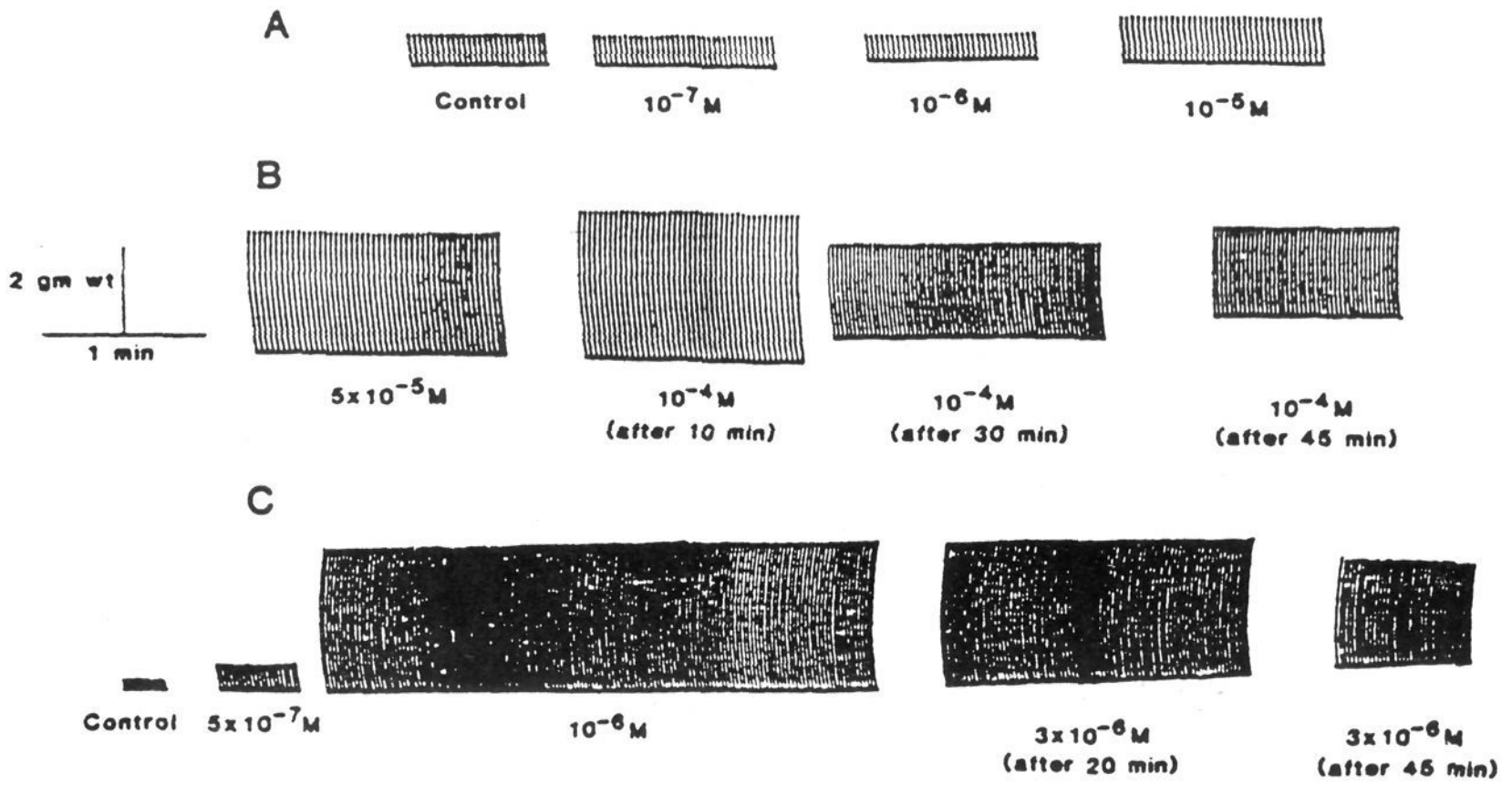


Figure 5. *Isometric contractions of right ventricular trabecula driven at a frequency of 0.5 Hz. Panel A, effects of cumulative addition* of the glucoside. Note increase in contractile force. Panel B, continuation of panel A. Note the initial increase followed by decrease in contractile force and the increase in diastolic tension at higher concentrations and with increased time of exposure to the glycoside. Mean increase in diastolic tension after 45 min exposure to glucoside was 1.43 ± 0.3 g (n = 6). Panel C, effect of cumulative addition of ouabagenin. Note the biphasic effect on contractile force. High concentrations elicit a rise in diastolic tension. Mean increase in diastolic tension after 45 min exposure to 3×10^{-6} M ouabagenin was 1.1 ± 0.24 g (n = 6).

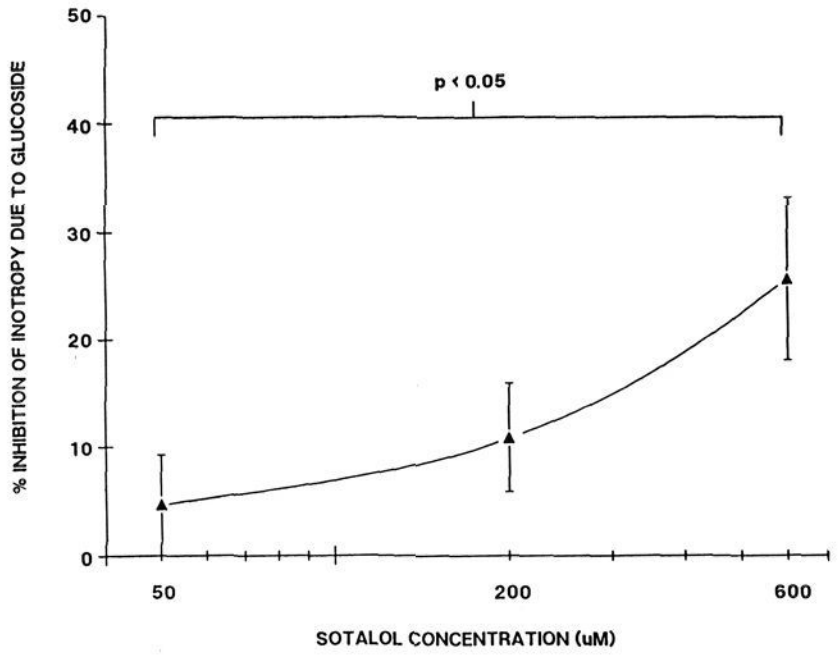


Figure 6. Dose–response curve of inhibition of the glucoside-induced inotropy by sotalol. Data are expressed as means ± SEM. Each point represents data from trabecular strips from three separate animals exposed to 10 μM of the glucoside.

Na,K-ATPase activity, have been studied in detail and, on the basis of several criteria, shown to interact at the cardiac glycoside binding site.

Conversion of 3β-alcohols **7** and **9** to the corresponding acetates **1** and **2** did not significantly alter their IC_{50} values in the ouabain binding assay (see Figure 1) whereas reduction of either **1** or **7** to the 20β-alcohol increased binding affinity.

Experimental Section

Steroid Synthesis. ^{1}H NMR spectra were recorded in deuteriochloroform on a Bruker AM 300 spectrometer. Column chromatography was carried out on silica gel (Merck type 60 H for TLC). Preparations were monitored by TLC on silica gel (Merck type 60H) plates in 25–75% ethyl acetate/hexane except for glucoside **5**, where 10% methanol/dichloromethane was used. Elemental analyses for carbon and hydrogen are within ±0.3% of the theoretical values.

3β-Acetoxy-14-hydroxy-5β,14β-pregnan-20-one (1). Hydrolysis[15] of digitoxin gave digitoxigenin, mp 247–255 °C (lit.[8] mp 253 °C), which on treatment with acetic anhydride and pyridine gave digitoxigenin acetate, mp 228–229 °C (lit.[9] mp 222–224 °C). The acetate (1.63 g) was in turn reacted with ozone, zinc in acetic acid, p-tosyl chloride, sodium iodide in acetone, and zinc in acetic acid according to the method of Baran[10] without isolation of intermediates to yield, after chromatography over neutral alumina and recrystallization from ethyl acetate/cyclohexane, 3β-acetoxy-14-hydroxy-5β,14β-pregnan-20-one (310 mg): mp 148–150 °C (lit.[11,26] mp 148–152 °C and mp 150–151 °C); ^{1}H NMR ($CDCl_3$) δ 0.97 (10- and $13CH_3$), 2.05 (3βOAc), 2.23 ($20CH_3$), 2.90 (dd, J = 4.3, 9.2 Hz, 17αH), 4.36 (14βOH), 5.08, ($W^{1/2}$ = 8 Hz, 3αH) ppm.

3β-Acetoxy-14,20β-dihydroxy-5β,14β-pregnane (2). A mixture of acetate **1** (680 mg) and LTBA (1.4 g) in dry ether (250 mL) was stirred at room temperature for 4 h when excess acetone was added followed by dilute HCl (20 mL, 5%). Extraction with ether followed by chromatography in dichloromethane and 1–5% acetone/dichloromethane mixtures over silica gave fractions which on recrystallization yielded the 20β-alcohol (420 mg): mp 167–169 °C, ^{1}H NMR ($CDCl_3$) δ 0.98 ($10CH_3$), 1.20 ($13CH_3$), 1.27 (d, J = 6.6 Hz, $21CH_3$), 2.05 (3βOAc), 2.45 ($W^{1/2}$ = 7.5 Hz, 14β- and 20βOH), 3.87 (m, 20αH), 5.08 (3αH) ppm. Anal. ($C_{23}H_{38}O_4$) C, H.

20β-[(*tert*-Butyldimethylsilyl)oxy]-3β,14-dihydroxy-5β,14β-pregnane (3). Diol acetate **2** (400 mg) was treated with t-$BuMe_2SiCl$ (400 mg) and imidazole (400 mg) in dry DMF (15 mL) as described by Corey[12] and the noncrystalline product (450 mg) [^{1}H NMR ($CDCl_3$) δ 0.11 (CMe_3), 0.92 ($SiMe_2$), 0.96 ($10CH_3$), 1.16 ($13CH_3$), 2.04 (3βOAc), 1.32 (d, J = 7 Hz ($21CH_3$), 3.86 (m, *20αH), 3.90 (14βOH), 5.06 (3αH) ppm] was treated in ether (100* mL) with LAH (100 mg) for 4 h when excess acetone was added and the mixture was acidified with dilute HCl and extracted with ether. Chromatography of the residue from evaporation of the ether over neutral alumina in dichloromethane gave fractions (240 mg) which on recrystallization from dichloromethane/acetone yielded 20β-silyloxy compound **3** (183 mg): mp 171–173 °C; ^{1}H NMR ($CDCl_3$) δ 0.12 (CMe_3), 0.92 ($SiMe_2$), 0.96 ($10CH_3$), 1.16 ($13CH_3$), 1.32 (d, J = 6.8 Hz, $21CH_3$), 3.87 (m, 20αH and ROH), 4.11 ($W^{1/2}$ = 12 Hz, 3αH) ppm. Anal. ($C_{27}H_{50}O_3Si$) C, H.

(26) Danielli, N.; Mazur, Y.; Sondheimer, F. *Tetrahedron* **1966**, *22*, 3189.

14,20β-Dihydroxy-3β-[(tetraacetoxy-β-D-glucopyranosyl)oxy]-5β,14β-pregnane (4). 3β-Tetraacetoxy-β-D-glucopyranoside **4** was prepared from 20β-silyl 3β-alcohol **3** (140 mg) by the method of Brown et al.[13] and the silyl group was removed by the method of Corey.[12] The crude product was purified by chromatography in dichloromethane over silica: elution with 10% acetone/dichloromethane yielded fractions (84 mg) which on recrystallization gave 3β-tetraacetoxy-β-D-glucopyranoside **4** (74 mg): mp 280–281 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 ($10CH_3$), 1.18 ($13CH_3$), 1.27 (d, J = 6.6 Hz, $21CH_3$), 2.008, 2.013, 2.020, 2.077 (2′-, 3′-, 4′- and 6′OAc), 2.52 (14β- and 20βOH), 3.67 (ddd, J = 2.5, 4.8, 9.9 Hz, 5′H), 3.85 (m, 20αH), 4.00 ($W^{1/2}$ = 7.5 Hz, 3αH), 4.11, 4.25 (J_{AB} = 12.2 Hz, $J_{A5'H}$ = 2.5 Hz, $J_{B5'H}$ = 4.8 Hz, 6′CH_2), 4.55 (d, J = 8 Hz, 1′H), 5.00 (dd, J = 8, 9.6 Hz, 2′H), 5.08 (t, J = 9.7 Hz, 4′H), 5.22 (t, J = 9.5 Hz, 3′H) ppm. C*H*OAc Assignments for the 3β-tetraacetoxy-β-D-glucopyranoside are based on homonuclear correlation (COSY spectrum analysis).[27] Anal. ($C_{36}H_{54}O_{12}$) C, H.

14,20β-Dihydroxy-3β-(β-D-glucopyranosyloxy)-5β,14β-pregnane (5). Hydrolysis of 3β-tetraacetoxy-β-D-glucopyranoside **4** (69 mg) by the method of Brown et al.[13] gave, after passing through silica in 10% methanol/dichloromethane and recrystallization from acetone/water, the 3β-(β-D-glucopyranoside) (46 mg): mp 199–201 °C; ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ 0.97 ($10CH_3$), 1.17 ($13CH_3$), 1.25 (d, J = 6.7 ($21CH_3$), 3.22–3.42 (m, 2′–5′H), 3.68–3.88 (m, 6′CH_2 and 20αH) 4.07 ($W^{1/2}$ = 7 Hz, 3αH), 4.33 (d, J = 7.7 Hz, 1′H) ppm. Anal. ($C_{27}H_{46}O_8 \cdot H_2O$) C, H.

3β,14-Dihydroxy-5β,14β-pregnan-20-one (7) and 3β,14-Dihydroxy-5β,14β,17α-pregnan-20-one (6). To a solution of 3β-acetoxy-14-hydroxy-5β,14β-pregnan-20-one (**1**, 100 mg) in methanol (18 mL) was added water (6 mL) followed by potassium hydrogen carbonate (100 mg) and the mixture refluxed in an argon atmosphere. After 5 h no starting material remained (TLC; 50% ethyl acetate/hexanes) and two more polar components appeared. TLC indicated conversion of the more polar component into the less polar component. The mixture was diluted with water (200 mL) and extracted with ether to give a residue [^{1}H NMR ($CDCl_3$) showed 63% 17αAc, 37% 17βAc by comparison of their 17H]. HPLC was carried out on a Waters μ-Porasil (10 μM) column in 1% ethanol/dichloromethane with a 1.5 mL/min flow rate (Waters M45 instrument and RI detector). HPLC separation gave, as the less polar component, 3β,14-dihydroxy-5β,14β-pregnan-20-one **7**, 29 mg) and the more polar component 3β,14-dihydroxy-5β,14β,17α-pregnan-20-one (**6**, 53 mg). **7**: mp 154–156 °C from dichloromethane/ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.96, 0.97 (10- and $13CH_3$), 2.23 ($21CH_3$), 2.90 (dd, J = 4.4, 9.2 Hz, 17αH), 4.12 ($W^{1/2}$ = 8 Hz, 3αH), 4.34 (14βOH) ppm. **6**: mp 191–192 °C from dichloromethane/ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 0.95 ($10CH_3$), 1.21 ($13CH_3$), 2.15 ($21CH_3$), 3.26 (t, J = 9 Hz, 17βH), 4.12 ($W^{1/2}$ = 8 Hz, 3αH) ppm. Anal. ($C_{21}H_{34}O_3$) C, H.

14-Hydroxy-5β,14β-pregnane-3,20-dione (8). 3β,14-Dihydroxy-5β,14β-pregnan-20-one (15 mg) was treated for 16 h at room temperature with pyridinium dichromate (80 mg) in dichloromethane (2 mL) containing dimethylforamide as described by Corey.[28] After addition of water followed by ether extraction, the residue was passed through neutral alumina in dichloromethane to give the 3,20-dione (10 mg): mp 196–198 °C from dichloromethane/ethyl acetate (lit.[14] mp 192–194 °C); ^{1}H NMR ($CDCl_3$) δ 0.98, 1.01 (10- and $13CH_3$), 2.23 ($20CH_3$), 2.33 (ddd, J = 5.3, 14.5, 14.8 Hz, 2αH), 2.64 (t, J = 14.7 Hz, 4αH), 2.91 (dd, J = 4.1, 9.2 Hz, 17αH), 4.43 (14βOH) ppm.

3β,14,20β-Trihydroxy-5β,14β-pregnane (9). To 3β-acetoxy-14,20β-dihydroxy-5β,14β-pregnane **2**, 40 mg) in dry ether (20 mL) was added LAH (40 mg). The mixture was stirred for 30 min at room temperature when excess acetone followed by water was added and the acidified (HCl) mixture was extracted with ether to give, after recrystallization from dichloromethane/methanol, 3β,14,20β-trihydroxy-5β,14β-pregnane (15 mg): mp 220–222 °C; ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ 0.97 ($10CH_3$), 1.17 ($13CH_3$), 1.26 (d, 6.5 Hz, ($21CH_3$), 3.79 (m, 20αH), 4.08 ($W^{1/2}$ = 7 Hz, 3αH) ppm. Anal. ($C_{21}H_{36}O_3$) C, H.

Biological Assays. [^{3}H]Ouabain Binding Assay. Hearts from pentobartital-anesthetized mongrel dogs were removed and immediately immersed in Krebs–Henseleit buffer [composition (mM): NaCl (118), KCl (4.7), CaCl (2.5), $MgSO_4$ (1.2), KH_2PO_4 (1.4), $NaHCO_3$ (26), glucose (11)] equilibrated with 95% O_2/5% CO_2 at room temperature. Within 60 min the ventricles were chopped with scissors and disintegrated by Polytron treatment (Brinkman Instruments Inc.) in ice-cold 50 mM Tris buffer, pH 7.4. The suspension was passed and the filtrate was centrifuged at 34000*g* for 45 min at 4 °C. The supernatant was discarded and the pellet resuspended in the buffer for radioligand-binding assay (see below) and stored at −20 °C. Once thawed, the tissue suspension was used immediately or discarded. The buffer was 45 mM Tris, 5 mM Tris phosphate, [^{3}H]ouabain in 0.1 mL of buffer, approximately 10 mg of tissue (wet weight) in 0.1 mL of buffer, and steroids (see below). Nonspecific binding (about 10% of total binding) was determined in the presence of 0.1 mM ouabain. Following incubation at 0 °C for 90 min, assay tubes were centrifuged at 2700*g* for 25 min in 0.3 mL of 2 N KOH and 1.2-mL aliquots were dispensed into vials for liquid-scintillation counting. Data was expressed as percent of specifically bound [^{3}H]ouabain (i.e., difference between total binding and nonspecific binding). The steroids dissolved in a few microliters of 95% ethanol were added to reaction tubes; this volume of ethanol alone had no effect on the specific binding of [^{3}H]ouabain. Similar potencies were obtained for the steroids in the radioligand-binding assay when the ethanolic solutions were evaporated to dryness in the reaction tube, tissue suspension was added, and the tube was vortexed for 1 min.

Measurement of Contractility of Isolated Cardiac Tissue. Dogs of either sex, weighing between 5 and 12 kg, were anesthetized with sodium pentobarbital (35 mg/kg ic), and the heart was removed. Thin (<1-mm diameter), free-running trabecula were tied to the base of a perspex holder which had embedded electrodes for punctate stimulation. The opposite end of the tissue was connected to a Grass FT-03C isometric force transducer and a Grass polygraph. A Pulsar 6i stimulator (F. Haer Co.) connected to a custom-built computer-controlled programmable pulse generator[30] provided square-wave stimuli of 3-ms duration to the trabecula. Stimulus amplitude was adjusted to about 10–20% above threshold. The resulting tension was increased until the maximum active tension in response to electrical stimulation was evoked. The basic stimulus interval was 2000 ms. Tissues were placed in a 10-mL vertical tissue bath containing Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2, and the bath temperature was maintained at 37.0 ± 0.2 °C. The muscle was equilibrated for 1 h prior to the experiment.

^{86}Rb Uptake. Human blood was collected from normal adult volunteers and placed on ice. Clotting was inhibited by 1 mM EGTA. The assay method was based on that of Belz[31] with minor modifications. Following preincubation with steroids at 37 °C for 60 min, RbCl (2 mCi mg^{-1}; NEN, Montreal) was added and incubation proceeded at 37 °C for 2 h. Duplicate assay tubes contained 0.3 mM RbCl [RbCl + 0.5 μCi ^{86}RbCl], 5.6 mM glucose, and drugs as required. Ouabain-insensitive ^{86}Rb uptake was determined in the presence of 0.1 mM ouabain. Cells were isolated by centrifugation, the radioactivity was determined, and the results were expressed as a percentage of that ^{86}Rb uptake which is inhibited by 0.1 mM ouabain.

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(27) Sanders, J. K. M.; Mersh, J. D. *Prog. Nucl. Magn. Reson. Spectrosc.* **1982**, *15*, 353.
(28) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1988**, *93*, 453.
(29) Bose, D.; Elliott, D.; Kobayashi, T.; Templeton, J. F.; Kumar, V. P. S.; LaBella, F. S. *Br. J. Pharmacol.* **1988**, *93*, 453.
(30) Boyechko, G.; Bose, D. *J. Pharmacol. Methods* **1984**, *12*, 45.
(31) Belz, G. G. In *Cardiac Glycosides*; Freef, K., Ed.; Springer-Verlag: Berlin, 1981; Part 1, pp 95–113.