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CARDIAC GLYCOSIDES: 3. SYNTHESIS OF β-D-DIGITOXOSE ANALOGUES

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Abstract--Earlier studies have shown that the β-D-digitoxose (2,6-dideoxy-β-D-mannopyranose) directly attached to the cardiac glycoside steroid C3 has the greatest effect on biological activity. This report describes the synthesis of eight digitoxosides (**2a-9a**), with widely varying cyclic and acyclic C17β-side groups, and the corresponding C3',C4'-acetonides (**2b-9b**). NMR analysis of conformational strain introduced by the acetonide groups is supported by crystallographic analysis of the sugars' torsion angles.

Cardiac glycosides[2] such as digoxin (**1a**) and digitoxin (**1b**) (Chart I) are currently among the six most-prescribed drugs in the U.S.[3] Their structures include three β-D-digitoxose (2,6-dideoxy-β-D-mannopyranose) sugars attached to the genin C3-O. Because of these glycosides' clinical and biochemical importance, we have been very interested in synthesizing new analogs and in delineating their pharmacological mechanisms of action. In studies with a variety of cardenolide genins, we found that the position of each side group's carbonyl oxygen (or in the case of a nitrile, its nitrogen) was a nearly perfect predictor of biological activity.[4] Most recently, we have identified the C17 side group receptor subunit on Na+,K+-ATPase using a site specific photoaffinity probe.[4f]

We have now turned our attention to a systematic study of the roles of structure and conformation of the C3 sugars; and in particular how they relate to the effects of modified C17β side groups.[1,5,6] The synthesis of β-D-digitoxides **2a-9a** was undertaken because, 1) their natural occurance of β-D-digitoxose in digitoxin and digoxin; 2) the 'first' digitoxose on digitoxin (i.e., the one directly attached to the genin C3) increases activity relative to digitoxigenin ten-fold; but the 'second' and 'third' digitoxoses have very little additional effect;[6,7] and 3) the possible clinical applications of a 'weak' C17β side group combined with a sugar which increases binding and activity. The genins were selected because of the structural variety of C17β side groups, and because they have over a 100-fold range in activities.[4] The acetonides **2b-9b** were also of interest because (in addition to being synthetic intermediates to **2a-9a**) they could be used in future biological studies to evaluate the roles of the 3' and 4' hydroxyls.

RESULTS AND DISCUSSION

Digitoxigenin β-D-digitoxoside (3β, 5β, 14β)-3-[2,6-dideoxy-β-D-ribohexopyranosyl) oxy]-14-hydroxycard-20(22)-enolide (**2a**) is the central intermediate in the synthesis of all the other β-D-digitoxosides reported in this study. It has previously been prepared in

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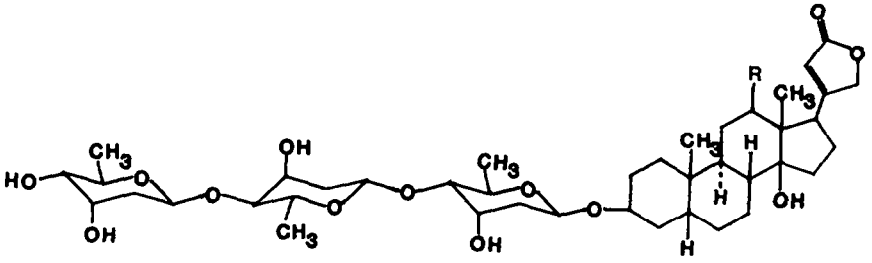
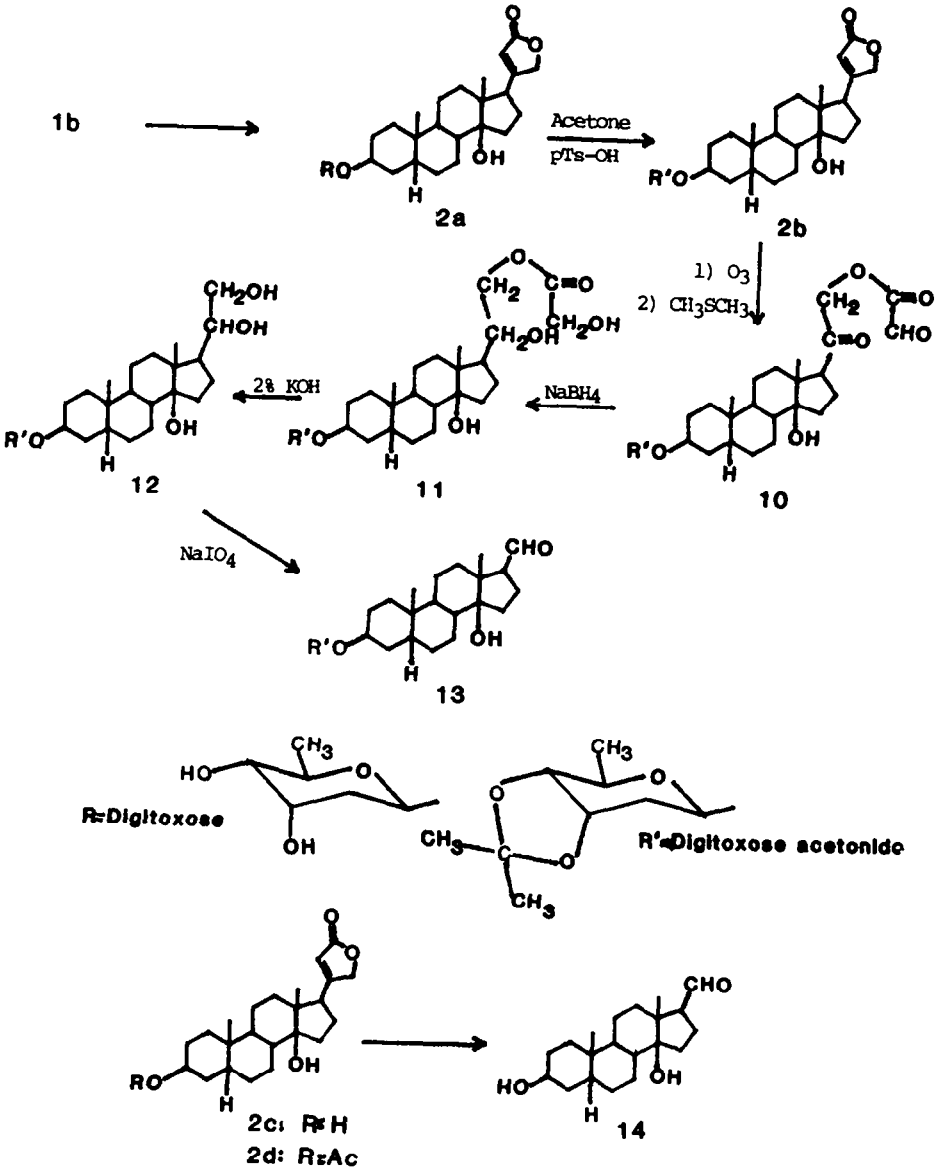
Chart 1



1a: R= OH

1b: R= H

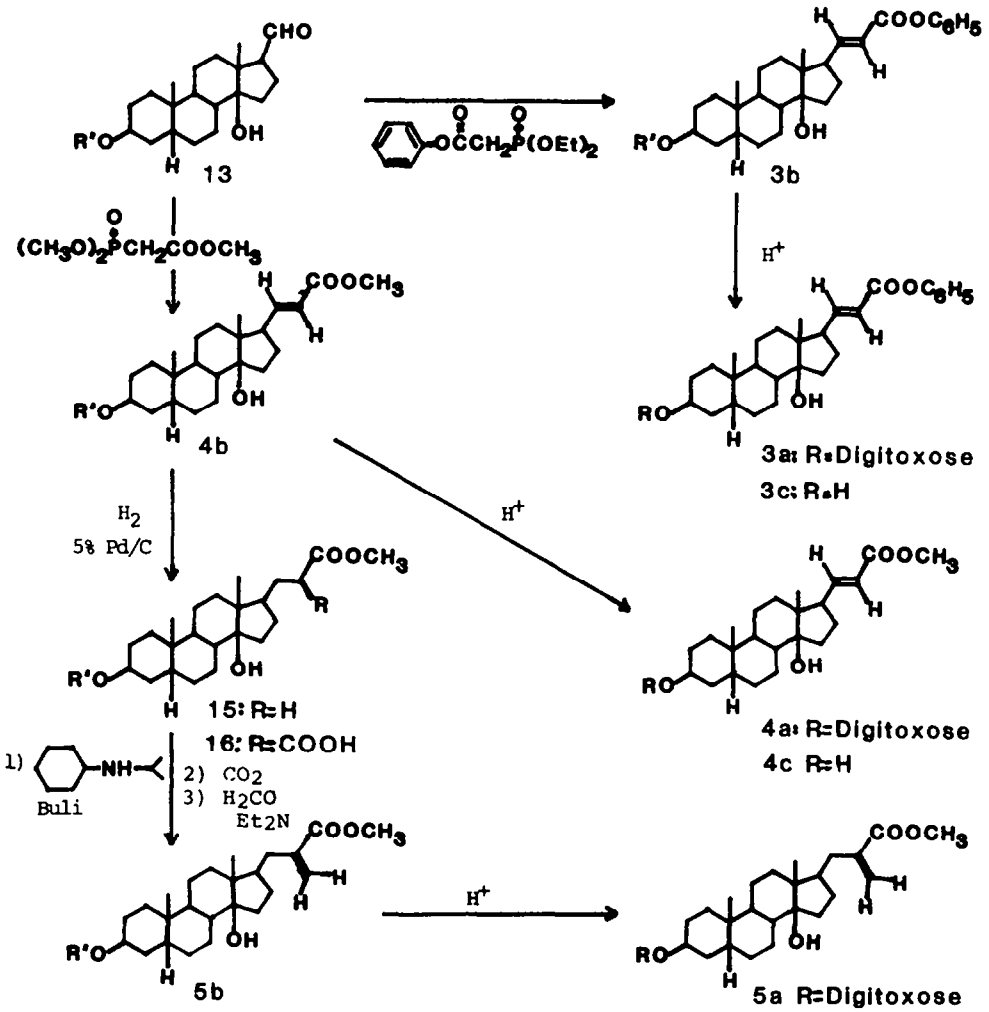


Scheme 1

small (10%) yield by Zorbach[8a] using acid catalyzed addition of digitoxose to digitoxigenin. Modified Koenigs-Knorr reaction of di-O-p-nitrobenzoyl digitoxose chloride with digitoxigenin was found by Zorbach[8b] to give the α-D-digitoxoside in 45% yield but little or no **2a**. The best yields of **2a** have been reported by Satoh and Aoyama[9] using a stepwise degradation of the two terminal digitoxoses of digitoxin (**1b**). The 3' and 4' hydroxyl groups of **2a** were then protected (acetone and p-toluenesulfonic acid) to give acetonide **2b** (3β,5β,14β)-3-2,6-dideoxy-3,4-O-(1-methylethylidine-β-D-ribo-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide) (Scheme 1).

Aldehyde **13** (3β,5β,14β,17β)-3-[(2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxyandrostane-17-carboxaldehyde) was prepared using a modification of a procedure used by us[10] and by Thomas[11] to convert digitoxigenin (**2c**) to its acetate **2d**, and then to the genin aldehyde **14**. Ozonolysis of **2b** followed by reduction of the ozonide with dimethylsulfide instead of zinc and acetic acid provided dialdehyde **10**, which was reduced with sodium borohydride to diol **11**. The hydrolysis of **11** with methanolic potassium hydroxyide and oxidation of the product **12** with sodium periodate gave aldehyde **13** in 72% yield overall yield from **2b**.

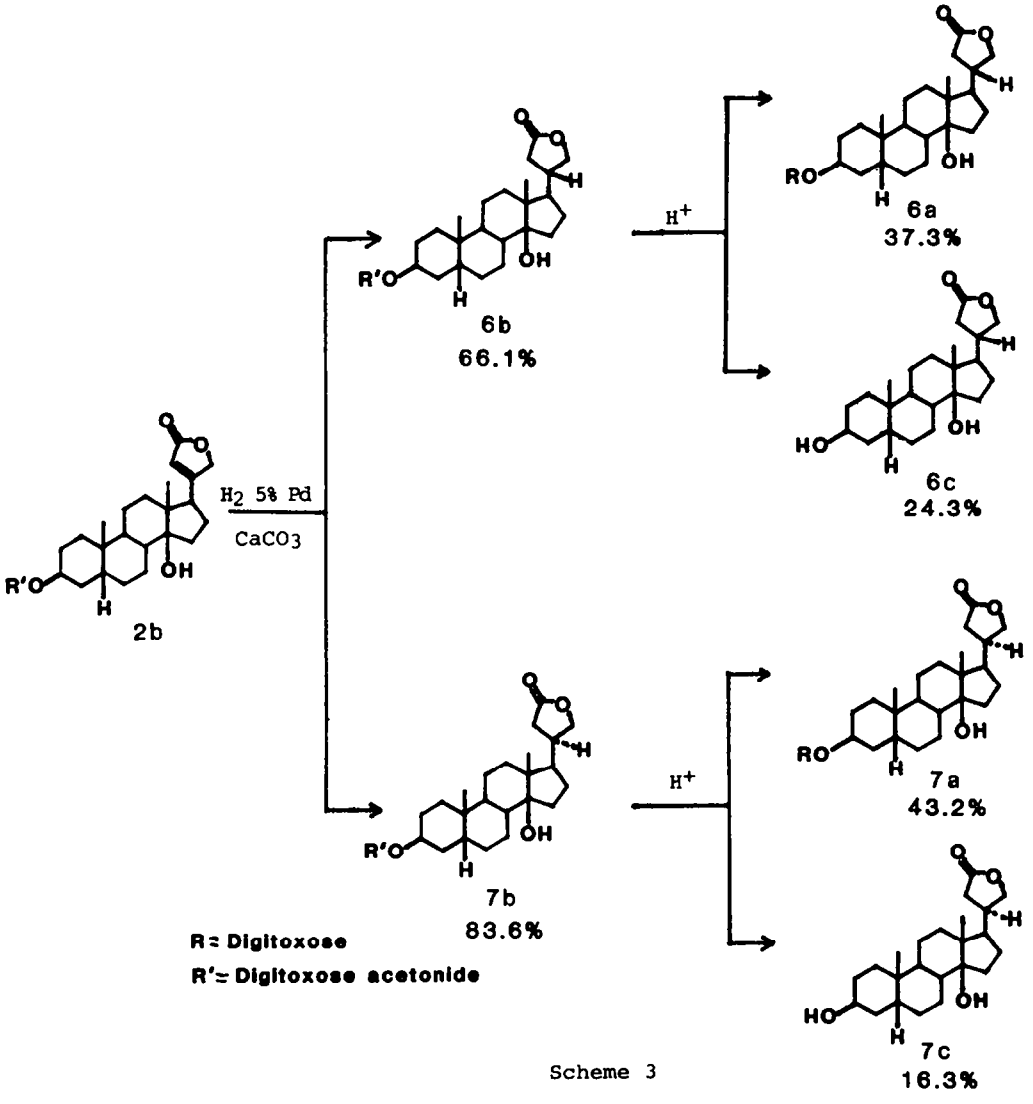
Wittig[12,13] reaction of **13** (Scheme 2) with phenoxycarbonylmethyl methyl phosphonate (prepared from phenyl bromoacetate and trimethyl phosphite (see Experimental Section)



Scheme 2

gave the phenoxy β-D-digitoxoside acetonide **3b** (3β,5β,14β,20E)-phenyl-3-[(2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl) oxy]-14-hydroxy-(pregn-20-ene-21-carboxylate) in 51% yield. Reaction of **13** with trimethyl phosphonoacetate--a modification of the procedure used by Thomas[11] and ourselves[1] to convert digitoxigenin **2c** to genin methyl ester **4c**--gave the methyl ester β-D-digitoxoside acetonide **4b** (3β,5β,14β, 20E)-methyl 3-[2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl-oxy]-14-hydroxyl-(pregn-20-ene-21-carboxylate) in 84% yield. Hydrolysis of the acetonide protecting groups (methanol, room temperature, trace of 5% HCl) invariably gave a small amount of the corresponding genin and unreacted acetonide as well as the desired β-D-digitoxoside. Hydrolysis of **4b** thus gave **4a** (48.2%) and **4c** (trace), along with unreacted **4b**; and **3b** gave **3a** (53.7%) and **3c** (30.4%) (Scheme 2). The PMR coupling constants (16 Hz) between the C20 and C21 protons of these compounds indicate the trans stereochemistry of the C20 double bond; and confirmed by x-ray crystallography of **4a**, **4b**, and **4c**[14]. All physical and spectral data (Tables 1 and 2) are in agreement with the assigned structures.

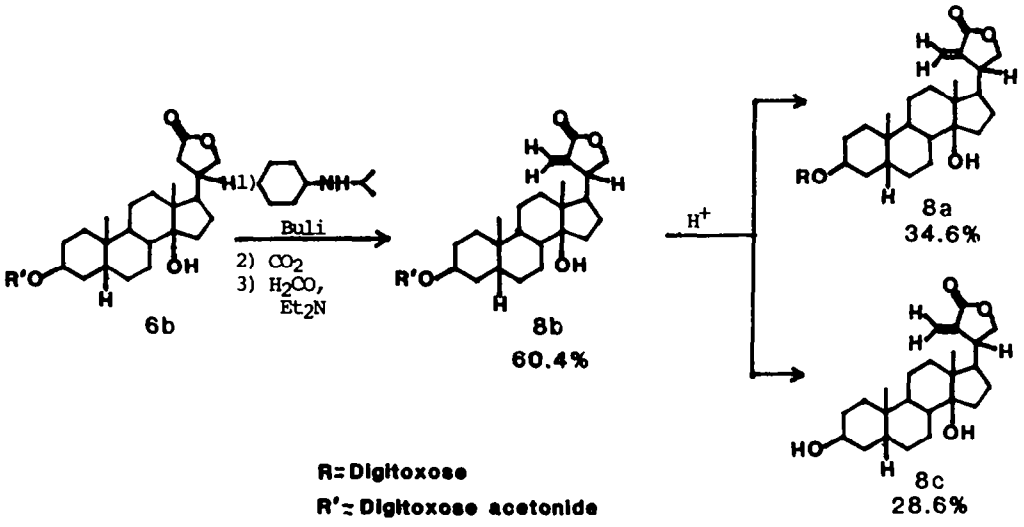
Catalytic hydrogenation of **4b** with 5% paladium on carbon as the catalyst[3e] gave dihydro compound **15**. The enolate of **15** was treated with anhydrous CO_2, followed by refluxing of the crude product **16** in aqueous formaldehyde and diethylamine (as previously reported by us for genins[3e]) to give acetonide **5b** (3β,5β,14β,20E)-methyl-3-[(2,6-dideoxy-3,4-

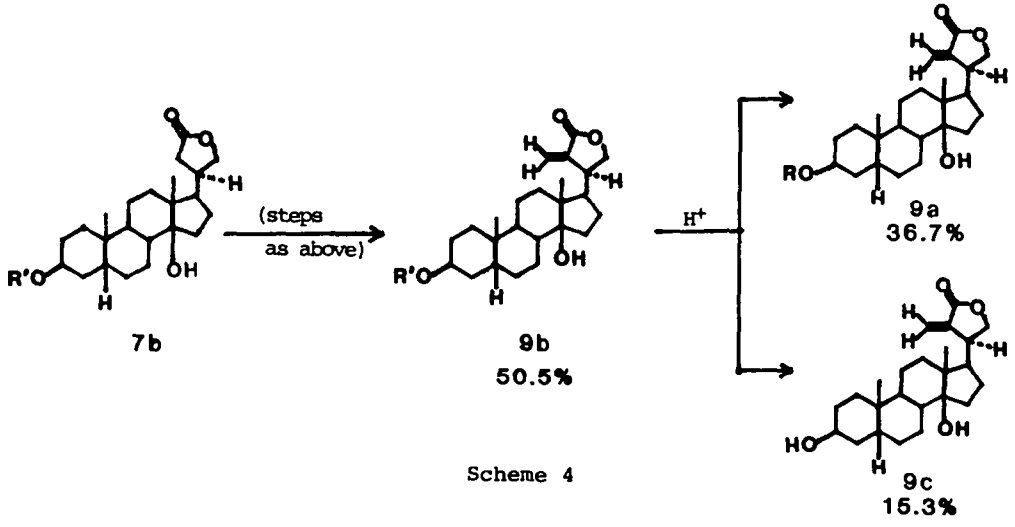


Scheme 3

O-(1-methylethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-21-methylene-(pregn-21-carboxylate) in 30% overall yield. Hydrolysis of **5b** gave **5a** (35.7%) along with recovered acetonide **5b**. We have previously reported that the (20R,S) mixture of genins (i.e., **6c** and **7c**) obtained by hydrogenation of digitoxigenin (**2c**) could be separated in good yield using fractional crystallization.[3e,15] We were not able to thus separate β-D-digitoxosides **6a** and **7a** (Scheme 3) but we could separate acetonides **6b** (20R) and **7b** (20S), 83.6% and 66.5% respectively from dihydro product **2b**, using a combination of methanol and ethyl acetate as a recrystallization solvent. The micro-TLC system developed[15] for the separation of the cardenolide diastereomers was also successful in separating **6a** and **7a**, an essential aid in following the progress of the fractional crystallizations. The chemical shifts and coupling constants δ 3.87(t, J=8 Hz) and δ 4.49(t, J=8 Hz) of the C21 protons of **6b** are clearly different from those of **7b**, δ 4.02(t, J=9 Hz) and δ 4.41(t, J=9 Hz) (Table 3). No trace of **7b** could be seen in the TLC nor in the PMR spectrum of **6b**; nor vice-versa for **6b**, providing good proof of diastereomeric purity. Absolute structural assignment of stereochemistry at C20, however, was provided by comparison with an authentic sample of **7b** and **7c** whose x-ray crystallographic analysis have already been completed.[4b,14]

22-Methylenation of **6b** and **7b** (Scheme 4) was completed in the same way used for **4b**, giving acetonides **8b** and **9b**, 50.5% and 60.4%





Scheme 4

overall yields, respectively. Subsequent hydrolysis gave β-D-digitoxoside **8a** and **9a**, along with the corresponding genins **8c** and **9c**. Absolute proof of C20 stereochemistry was on the basis of spectral comparison with our sythentic samples of **8c** and **9c**[3e]--and an x-ray crystal structure has been completed on the authentic sample of **8c** and **9b**.[4b,14] Although the PMR and ^{13}C-NMR spectra of some other digitoxigenin mono-glycosides have been reported[11b,17,18] they have not been for digitoxosides nor their acetonides. The protons (Table 2) of the steroid skeleton were easily assigned by comparing chemical shifts and coupling constants with those of the corresponding genins.[4e] Assignment of the sugar protons of the methyl ester acetonide **4b** was completed by decoupling experiments. Irradiation of C6'-H (δ 1.16,d,J= 6Hz) gave a doublet (J=9 Hz) for C5'-H (δ 3.44), and octet before decoupling. The octet (J=9 and 6 Hz) of C5'-H was irradiated to collapse C6'-H to a singlet. The irradiation of C2'-H (δ 2.07) gave a doublet (J3'-4'=5 Hz) for C3'-H (δ 4.40) and singlet C1'-H (δ 4.77). Irradiation of C3'-H (δ 4.40) produced a doublet (J=9 Hz) for C4'-H (δ 3.76). The assignment of the PMR spectra for the other compounds' sugars was then made on the basis of those of **4b** (see Table 2). The conditions used during the synthesis did not affect the anomeric (C1') β stereochemistry from the original starting material digitoxin (**2b**). The C1' proton of each digitoxoside has a doublet of doublets PMR absorption, J=9 or 8 Hz and J=2 or 3 Hz. This absorption is in good agreement with the natural digitoxoside **2a**. Although we would not expect a change in the anomeric carbon under the conditions used in the work, it should be noted that if α-anomers were present, the C1' coupling constants should be 3 Hz or less.[19]

It may be noted in Table 2 that the C1' protons in the acetonides are at higher field than those for the free digitoxosides. The coupling constants (J=6 Hz) between C3'-H and C4'-H in the acetonides are also larger than that (J=5 Hz) of the free digitoxosides. These data suggest that the pyranose rings in the acetonides are distorted by the protecting group itself.

The crystal structures of five digitoxosides (**4a**[14], gitoxin,[20] digoxin,[21] and the mono[22] and digitoxosides[23] of digoxin) and five digitoxose acetonides (**2b**,**4b**,**5b**,**7b**, and **9b**[14]) provide the structural data to substantiate the conclusions drawn from the spectral results. The averaged structural parameters for the sugar moiety in each group show a high degree of internal consistency. Comparison of the corresponding averaged parameters for the

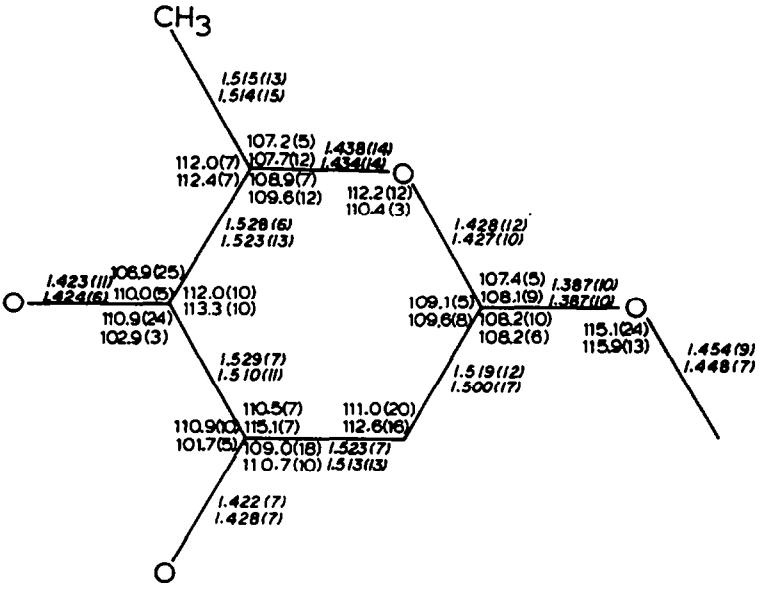


Figure 1

Average bond lengths and bond angles for the digitoxoside portion of cardiac glycoside analogs containing a digitoxoside, top values, or a digitoxose acetonide, bottom values. The numbers in parentheses are the standard deviation of the average.

two groups shows that there are no significant differences in the bond lengths, but that there are four bond angles which show significant variations: C2'-C3'-C4', C4'-C3'-O3', C3'-C4'-O4' and C5'-C4'-O4'. However, only the C2'-C3'-C4' angle is in the hexopyranosyl ring, the other three angles are all exocyclic (see Fig. 1).

The conformation of the hexopyransoyl ring is significantly flattened by the acetonide group. The C1'-C2'-C3'-4' and C2'-C3'-C4'-C5' torsion angles are distorted by 12.5° and -13.4° respectively in going from the free digitoxoside to the digitoxose acetonide. All of the torsion angles involving O3' and O4' are also significantly altered by the presence of the acetonide, see Table 3. Thus, the spectral results and the crystal structure data are in perfect agreement.

Hog kidney Na+,K+-ATPase inhibitory studies have been completed on the digitoxosides and acetonides described in this paper.[14] The digitoxosides were consistantly about an order of magnitude more active than the corresponding genins, but the acetonides were only about twice as active as the genins. The glycosides carbonyl oxygen positions were also shown to correlate very well with those of the genins. The contributions of the sugars to Na+,K+-ATPase inhibitory activity is quite separate, confirming the proposal first made by Yoda that the Na+,K+-ATPase receptor has distinct sugar and sugar binding sites.

TABLE 1. PHYSICAL DATA OF DIGITOXOSIDES

		ELEMENTAL ANALYSIS		IR SPECTRA (cm-1):KBr			$[\alpha]_D^{22}$ (c)
	FORMULA	C CALCD. (FOUND)	H CALCD. (FOUND)	OH	C=O	C=C	in MeOH
2a	C29H4407	69.02 (69.35)	8.78 (9.00)	3450 (br)	1725(br) 1775(sh)	1620	-5.6°a)(0.305)
2b	C32H4807	70.56 (70.60)	8.88 (9.00)	3500	1740	1625	+8.5°(0.270)
3b	C37H5207	73.00 (73.81)	8.61 (8.64)	3440 (br)	1720	1640	+27.7°(0.300)
4b	C32H5007	70.30 (70.12)	9.22 (8.96)	3250 (br)	1710	1650	+18.7°(0.300)
3a	C34H4807	71.80 (71.68)	8.51 (8.78)	3400 (br)	1720	1640	+13.7°(0.300)
4a	C29H4607	68.75 (68.54)	9.15 (9.05)	3550 3420(bd)	1720	1640	+10.0°(0.300)
5b	C33H5207	70.68 (70.83)	9.25 (9.37)	3500(br)	1720	1630	+10.0°(0.310)
5a	C30H4807	69.20 (69.26)	9.29 (9.30)	3440(br)	1710	1630	-2.6°(0.350)
6a	C29H4607	68.75 (68.55)	9.15 (9.00)	3480(br)	1770		-4.3°(0.305)
7a	C29H4607	68.75 (69.00)	9.15 (8.89)	3500(br) 3380(br)	1775(sh) 1750		-10.6°(0.265)
6b	C34H5007	70.30 (70.10)	9.22 (0.29)	3500(br)	1775		+12.1°(0.214)
7b	C32H5007	70.30 (70.57)	9.22 (8.95)	3520	1780 1760		+2.6°(0.232)
8b	C33H5007	70.94 (71.11)	9.02 (8.94)	3510	1750	1656	-49.5°(0.275)
9b	C33H5007	70.94 (71.15)	9.02 (8.93)	3490	1750	1660	-79.3°(0.275)
8a	C30H4607	69.47 (69.62)	8.94 (8.74)	3440(br)	1750	1660	-64.8°(0.290)
9a	C30H4607	69.47 (69.43)	8.94 (8.92)	3500(br)	1750	1660	+69.5°(0.220)
13	C29H4606	70.99 (70.92)	9.45 (9.65)	3470	1705		-14.7°(0.300)
15	C32H5207	70.04 (70.21)	9.55 (9.60)				+14.8°(0.330)

a) D. Satoh and K. Aoyama reported -5.2° (c = 0.327, MeOH)[1]

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, Ariz. The 100-MHz PMR were taken in $CDCl_3$ at the Oregon State University Department of Chemistry Spectroscopy Laboratory. IR spectra were run as KBr pellets or NaCl films (for liquids) using a Beckmann Model Acculab 7 spectrophotometer. Optical rotations in methanol were taken on a Perkin-Elmer 141 polarimeter. Ozonolysis was performed using the Ozonator Model 03V10-0 from the Ozone Research and Experiment Corporation (OREC).

Dry column chromatogrpahies[17] (as developed by K. Yoshioka and coworkers at Takeda Research Laboraories but unpublished, and modified by us) were completed using EM silica gel 60 (70-230 mesh) in a Buchner funnel with medium porosity fritted disk, 15 ml funnel for 50-100 mg compound; 60 ml funnel for 100-500 mg compound; 150 ml funnel for 500 mg - 2 g compound; with 10, 30, and 50 g silica gel respectively. The compound (or crude product) is dissolved in appropriate solvent (usually methylene chloride) having the same volume as the amount of silica gel used. (In column chromatography, the goal is normally to dissolve the compound in as little solvent as possible.) Subsequent elution of compounds was with the same solvent system used for thin layer chromatography (described below).

Thin layer chromatographies were on microscope slide sized .25 mm EM silica gel 60 F-254 as previously described.[15] Solvent combinations used were: "A" CH_2Cl_2 20: EtOAc 2: MeOH 1; "B" CH_2Cl_2 10: EtOAc 3: MeOH 1. Preparative chromatographies were on plates coated by us with EM silica gel 60 PF-254 .75 mm. Analytical tlc plates were visualized by UV light at 254 nm, iodine vapor, or by spraying with 2% $CeSO_4$ in 2N H_2SO_4 and heating in a hood on a hot plate.

Spectral, analytical, and optical rotation data for final compounds are shown in Tables 1 and 2.

(3β,5β,14β)-3-[2,6-dideoxy-3.4-O-(1-

TABLE 2. PMR SPECTRA[a,b] OF DIGITOXIDES IN $CDCl_3$ (δ)

	C1'-H	C2'-H	C3'-H	C4'-H	C5'-H[c]	C6'-H	C3-H	C20-H	C18-H[d]	C19-H[d]
2b	4.78 (dd,9,3)		4.42 (m)	3.68 (dd,8,5)	3.44 (m,8,6)	1.24 (d,6)	4.03 (m)		0.86	0.92
2a	4.89 (dd,9,3)		4.12 (m)	3.73 (dd,9,6)		1.28 (d,6)	4.04 (m)		0.88	0.93
3b	4.79 (dd,8,3)		4.42 (m)	3.68 (dd,9,5)	3.46 (m)	1.24 (d,6)	4.07 (m)		0.19	0.92
3a	4.90 (dd,9,3)		4.12 (m)	3.73 (dd,9,5)	3.37 (m)	1.25 (d,6)	4.08 (m)	7.28 (dd,16,10)	0.90	0.92
4b	4.77 (dd,9,3)	2.07(m) 2.18-1.98	4.40 (m)	3.67 (dd,9,5)	3.44 (m,9,6)	1.16 (d,6)	4.03 (m)	7.17 (dd,16,10)	0.87	0.93
4a	4.88 (dd,9,3)	2.02(m)	4.11 (m)	3.66 (dd,9,6)	3.45 (m,9,6)	1.29 (d,6)	4.02 (m)	7.18 (dd,16,10)	0.85	0.92
5a	4.88 (dd,8,2)		4.11 (m)	3.66 (dd,9,6)	3.37 (m,9,6)	1.28 (d,6)	4.02 (m)		0.93	0.98
5b	4.77 (dd,8,3)		4.43 (m)	3.65 (dd,9,5)	3.45 (m,9,6)	1.26 (d,6)	4.03 (m)		0.95	1.00
6b	4.76 (dd,8,3)		4.41 (m)	3.66 (dd,9,5)	3.44 (m,9,6)	1.24 (d,6)	4.01 (m)		0.94	0.94
6a	4.86 (dd,8,2)		4.09 (m)	3.71 (dd,9,6)	3.33 (m,9,6)	1.27 (d,6)	4.02 (m)		0.91	0.93
7b	4.76 (dd,8,3)		4.39 (m)	3.66 (dd,9,5)	3.43 (m,9,6)	1.24 (d,6)	3.99 (m)		0.92	0.96
7a	4.86 (dd,8,3)		4.11 (m)	3.72 (dd,9,6)	3.36 (m,9,6)	1.28 (d,6)	4.02 (m)		0.92	0.97
8b	4.75 (dd,9,3)		4.39 (m)	3.66 (dd,9,5)	3.42 (m,9,6)	1.23 (d,6)	4.00 (m)	3.25 (m)	0.92	0.98
8a	4.75 (dd,9,2)		4.13 (m)	3.73 (dd,9,6)	3.37 (m,9,6)	1.28 (d,6)	4.02 (m)	3.27 (m)	0.93	0.99
9b	4.77 (dd,9,3)		4.41 (m)	3.67 (dd,9,5)	3.44 (m,9,6)	1.23 (d,6)	4.01 (m)	3.29 (m)	0.90	0.91
9a	4.87 (dd,9,2)		4.12 (m)	3.73 (dd,9,6)	3.36 (m)	1.28 (d,6)	4.03 (m)	3.28 (m)	0.90	0.91
13	4.73 (dd,9,3)		4.37 (m)	3.60 (dd,9,5)	3.39 (m)	1.16 (d,3)	3.97	9.7	0.93	0.85
15	1.77 (dd,8,3)		4.41 (m)	3.66 (dd,9,5)	3.44 (m,9,6)	1.29 (d,6)	4.01 (m)		0.92	0.95

a) m=multiplet, dd=doublet of doublets.
b) Coupling constants in Hz are in ( ).
c) Each is an octet.
d) Singlet

methylethylidine)β-D-ribo-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (**2b**). Digitoxigenin monodigitoxoside mp 200-202° (**2a**) was prepared from digitoxin using the method of Satoh.[9] It (812 mg, 1.6 mmol) was then dissolved in acetone (30 ml). To this solution was added 8 mg of paratoluenesulfonic acid with stirring at room temperature for 3 hr. The reaction mixture was concentrated to half the volume in vacuo at 50°C. Water was added to the residue and this was extracted three times with CH_2Cl_2. The CH_2Cl_2 layer was washed with 5% $NaHCO_3$ and with water. After drying over $MgSO_4$, the solvent was evaporated in vacuo to afford 900 mg of crude oil of **3b**, which was purified using dry column chromatography and preparative TLC using solvent system A. The main product, Rf = 0.65, was extracted with CH_2Cl_2 - MeOH - acetone (1:1:1) to give 806 mg of pure oil which was crystallized by acetone-hexane to afford 650 mg (74%) of **2b**, mp 202-206°.

(3β,5β,14β,17β)-3-[(2,6-dideoxy-3,4-O-(1-methylethylidine-β-D-ribo-hexopyranosyl)oxy]-14-hydroxyandrostane-17-carboxaldehyde (**13**). Acetonide **2b** (2 g, 3.67 mmole) was dissolved in 100 ml of CH_2Cl_2 and cooled at -78° with a dry-ice bath. This solution was ozonized for 5 hr to afford a deep purple color characteristic of the ozonide. After removing of excess ozone by flushing with nitrogen, to the clear ozonide solution was gently added 4 ml of dimethylsufide and allowed to stand overnight raising from -78° to room temperature as the dry-ice sublimed. The reaction mixture was washed three times with H_2O. The methylene chloride layer was dried over $MgSO_4$, filtered and evaporated in vacuo to yield a crude powder of **10** (2 g), which was shown on TLC using solvent system B to be a single spot. The crude **10** was then dissolved in 100 ml of MeOH. To this solution was slowly added 316 mg (8.35 mmol) of $NaBH_4$ and stirred at room temperature for 1 hr. The reaction mixture was evaporated in vacuo to half the volume, H_2O was added and the mixture was extracted three times with ether. The ether extract was washed with water and with saturated NaCl, dried over $MgSO_4$, and evaporated in vacuo to 2.46 g of crude oil **11**. This was

TABLE 2 CONTINUED

	C21-H		C22-H	CH2 =	CH3 of Acetonide[d]
	Ha	Hb			of $COOCH_3$
2b	5.04 (dd,18,2)	4.78 (dd,18,2)	5.88(m) Wh/2 3Hz		
2a	5.03 (d,18)	4.79 (d,18)	5.87(m) Wh/2 3Hz		
3b	5.00 (d,16)				1.45,1.33
3a	5.89 (d,16)				
4b	5.62 (d,16)				1.58,1.36 3.71
4a	5.63 (d,16)				 3.70
5a				6.12(m,Wh/2 3Hz) 5.53(m,Wh/2 3Hz)	 3.73
5b				6.12(m,Wh/2 3Hz) 5.53(m,Wh/2 4Hz)	1.49,1.36 3.75
6b	3.87 (t,8)	4.49 (t,8)			1.46,1.34
6a	3.87 (t,8)	4.49 (t,8)			
7b	4.02 (t,9)	4.41 (t,9)			1.46,1.34
7a	4.03 (t,9)	4.41 (t,9)			
8b	4.18 (dd,9,3)	4.39 (dd,9,7)		6.26(d,2) 5.69(d,2)	
8a	4.19 (dd,9,3)	4.41 (dd,9,7)		6.26(d,2) 5.72(d,2)	1.46,1.34
9b	4.35 (dd,10,8)	4.70 (dd,10,6)		6.25(d,3) 5.53(d,3)	
9a	4.36 (dd,10,8)	4.70 (dd,10,6)		6.28(d,3) 5.52(d,3)	
13					1.39,1.27
15					1.47,1.35 3.75

shown on TLC in solvent system B to have two close spots due to the epimers at C20. The crude oil of **11** was dissolved in a solution of 2% KOH/MeOH (100 ml) and refluxed for 30 min on a steam bath. After cooling to room temperature, the reaction mixture was rotoevaporated to half the volume and the pH of the solution was adjusted between 5-6 with glacial acetic acid. Water was added slowly and the crystals formed were filterated after cooling in an ice bath. The crude crystals were washed with water, dried under vacuum to afford 1.4 g of **12**. To a solution of **12** (1.4 g) in MeOH (60 ml) was added $NaIO_4$ (1.4 g) in H_2O (14 ml) and stirred at room temperature for 1 hr. The salt of the $NaIO_3$ formed was filtered and washed with MeOH. The methanol filtrate was concentrated to half the volume in vacuo and water was added to the residue. The milky solution thus obtained was cooled in ice bath. The resulting crystals were collected by suction, washed with water and dried to afford 1.3g (72%) of **13**, mp 75-80°.

(3β,5β,14β,20E)-phenyl-3-[2,6-dideoxy-3,4-O-(1-methyl-ethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-(pregn-20-ene-21-carboxylate) (**3b**).

a) Synthesis of phenoxycarbonylmethyl diethyl phosphonate. Phenyl bromoacetate: Bromoacetyl bromide (50 g) was added dropwide (30 min) to an ethanol solution (400 ml) of phenol (23.5g) and pyridine (22 g), and the solution was stirred in a water bath for 1 hr at room temperature. After the pyrinine-HBr salt was filtered off, the filtrate was concentrated in vacuo to give a crude oil which was separated by dry column chromatography using solvent system A. The product with Rf 0.85 was distilled under reduced pressure to yield 13.5g (25%) of phenyl bromoacetate, bp 134-138°/15 mm Hg. After cooling at -20°, the oil gave crystals, mp 32°, IR(NaCl film) 1750 (C=O), 1590 and 149° (aromatic) cm^{-1}; NMR ($CDCl_3$, 60 MHz) δ 4.0 (s, 2H, $COCH_2Br$), 7.2 (m, 5H, aromatic).

Synthesis of the phosphonate: Phenyl bromoacetate (13 g, 60.45 mmol) was mixed with 14.1 g (84.86 mmol) of triethyl phosphite and refluxed at 155° on an oil bath for 1 hr with generation of ethyl bromide. The crude oil was distilled to yield 14.4 g of pure phosphonate, bp 150-153°/.2 mmHg. (Lit[13] bp 174-176° 1.25 mmHg). IR (NaCl): 1750 (C=O), 1595, 1500 (aromatic), 1250(-PO-) cm^{-1}; NMR ($CDCl_3$, 60 mHz) δ: 1.38 (t, 3H, OCH_2CH_3), 3.18 (d, J=23Hz, $-COCH_2PO-$) 4.13 (m, 2H, OCH_2CH_3), 7.25 (m, 5H, aromatic).

b) Synthesis of 3b: To sodium hydride (50% in oil, 9.8 mg, 2.03 mmole, washed twice with hexane) was added 3 ml of dry diglyme. The phosphonate (520 mg, 2.03 mmol) in dry diglyme (5 ml) was then added at room temperature under nitrogen. After stirring for 15 min, **13** (400 mg. 0.81 mmol) dissolved in 5 ml of dry diglyme was added and stirred for 1.5 hr. The reaction mixture was diluted with large excess of acetate buffer (pH 5-6), then cooled in ice. The crystals were filtered and dissolved in CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to yield a crude oil (504 mg), which was separated by dry column chromatography with solvent system A to give 305 mg of pure oil. This was converted to a powder by 'crystallization' in hexane, to yield 250 mg (51%) of **3b**, mp: 82-84.

(3β,5β,14β,20E)-phenyl-3-[(2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14-hydroxypregn-20-ene-21-carboxylate (**3a**). To a solution of **3b** (160 mg, 0.262 mmol) in MeOH (50 ml) was added 2 drops of 5% HCl. This was stirred at room temperature for 6 hr. The reaction mixture was poured into water and extracted three times with CH_2Cl_2. The methylene chloride layer was washed with water, dried over $MgSO_4$, and evaporated in vacuo to give an oil. The crude oil was separated by dry column chromatography and preparative TLC to afford 41 mg (25.6%) of starting material **3b** from the band of Rf 0.94. The extraction of Rf 0.49 gave 35

Table 3: AVERAGE TORSION ANGLES OF DIGITOXOSIDES VS ACETONIDES

Ave. Torsion Angles in Degrees (standard deviation)

Atoms	Digitoxoside	Acetonide	Change
C1'-C2'-C3'-C4'	-50.1(2.0)	-37.5(2.2)	12.6
C2'-C3'-C4'-C5'	49.0(1.2)	35.6(2.2)	-13.4
C3'-C4'-C5'-O5'	-54.3(2.5)	-48.3(4.7)	6.0
C4'-C5'-O5'-C1'	63.1(2.2)	65.7(2.4)	2.6
C5'-O5'-C1'-C2'	-65.1(3.2)	-68.2(2.5)	-3.1
O5'-C1'-C2'-C3'	57.4(3.8)	52.5(4.6)	-4.9
O3-C1'-C2'-C3'	174.0(3.6)	170.1(5.3)	-3.9
O3-C1'-O5'-C5'	177.8(3.7)	174.1(3.0)	-3.7
O3'-C3'-C2'-C1'	72.0(1.0)	77.1(2.8)	5.1
O3'-C3'-C4'-C5'	-72.0(2.3)	-84.1(2.5)	-12.1
O3'-C3'-C4'-O4'	47.3(2.7)	34.6(2.4)	-12.7
O4'-C4'-C3'-C2'	168.3(2.4)	154.3(2.7)	-14.0
O4'-C4'-C5'-O5'	-175.9(2.5)	-162.8(4.4)	13.1
O4'-C4'-C5'-C6'	65.6(2.2)	77.5(5.2)	11.9
C6'-C5'-C4'-C3'	-172.7(2.4)	-168.0(5.6)	4.7
C6'-C5'-O5'-C1'	-175.4(2.5)	-171.8(3.0)	3.6

mg (30.4%) of genin **3c**. The main product **3a** (80 mg, 53.7%, mp 103-105) was obtained from the band at rf 0.37 as a powder and crystallization from hexane.

(3β,5β,14β,20E)-methyl-3-[2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-(pregn-20-ene-21-carboxylate (**4b**). Methyl ester **4b** was synthesized from **13** (245 mg, 0.5 mmole) in diglyme 2.5 ml, trimethylphosphnoacetate (228 mg, 1.25 mmole) and NaH (60 mg of 50% oil, 1.25 mmole) in diglyme 1.5 ml in a similar manner to that of **3b** described above. The crude product was recrystallized from n-hexane-CH_2Cl_2 to give white needles of **4b** (230 mg, 83.9%) mp 147-149°.

3β,5β,14β,20E)-methyl-3-[(2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14-hydroxypregn-20-ene-21-carboxylate (**4a**). A solution of **4b** (125 mg) in 100 ml of MeOH and 2 drops of 5% HCl was stirred for 5 hr. The reaction mixture was treated in a same way as that of **3b** to afford crude product of **4a**, which was purified by dry column chromatography using EtOAc-CH_2Cl_2 (9:1 to 2:8). The starting material **4b** (34 mg, 27.2%, mp 147-149°) was recovered from first fraction. The second fraction gave 72 mg (62.1%) of **4a** as an oil, which was recrystallized from EtOAc hexane to yield pure crystals (56 mg, 48.2%), mp 191-193°. A trace of genin **4c** which was also seen on TLC, same Rf as **4c** we have previously reported, but it was not isolated.

(3β,5β,14β,17β)-methyl-3-[(2,6-dideoxy-3,4-O-(1-μmethylethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-(pregn-21-carboxylate) (**15**). The unsaturated ester **4b** (541 mg) in MeOH 100 ml was hydrogenated (1 atm) with 5% Pd-C (100 mg) for 5 hr at room temperature. The filtration and evaporation of the reaction mixture afforded a powder (563 mg). This was recrystallized from CH_2Cl_2-hexane to give 425 mg (78.2%) of **15**, mp 135-136°.

(3β,5β,14β)-methyl-3-[(2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-21-methylene-(pregn-21-carboxylate) (**5b**). To an ice cooled solution of N-isopropylcyclohexylamine 0.5 ml (0.486 g, 277 mmol) in anhydrous THF 2.5 ml was added 1.7 ml (2.72 mmol) of 1.6 M butyl lithium in hexane under N_2. The solution was stirred for 10 min and then at -78° for 20 min. To the solution was added 200 mg (0.364 mmol) of **15** in anhydrous THF 2.5 ml and stirred for 40 min. Anhydrous CO_2 (Matheson, Bone-Dry grade) was bubbled into the solution for 4 min and the dry-ice bath was removed. Bubbling continued for 25 min, during which time the solution warmed to room temperature. The reaction mixture was poured into 15 ml of 5% HCl and extracted three times with ether, washed with water, dried over $MgSO_4$, and evaporated to give a crude product, presumably **16**. To this crude product was added 3 ml of 37% formalin (with 10% MeOH) and 0.8 ml of diethylamine. The solution was heated on a steam bath for 30 min. The reaction mixture was cooled in an ice bath and to it was added 1 ml of 5% HCl and 9 ml of water. The mixture was extracted three times with ether. The ether layer was washed with water, dried over $MgSO_4$, and evaporated in vacuo to afford an oil. The oil was purified by dry column chromatography using CH_2Cl_2-AcOEt and recrystallized from CH_2Cl_2-hexane to give 61 mg (29.9%) of **5b**, mp 165-167°.

3β,5β,14β,20E)-methyl-3-[(2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14-hydroxy-21-methylene-(pregn-21-carboxylate) (**5a**). A solution of **5b** (75 mg) in MeOH 50 ml was stirred with one drop of 2% HCl at room temperature for 75 hr. The reaction mixture was treated in the same way as that of **3b** to give a powder (58 mg), which was purified by preparative TLC (solvent system B). The oily product (33 mg) obtained from the band at Rf 0.50-0.56 was recrystallized from EtOAc-hexane to yield 25 mg (35.7%) of **5a**, mp 172-174°. Starting material **5b** (10 mg, mp 165-166°) was recovered from the band Rf 0.86-0.95.

(20R and 20S)-20,22-dihydrodigitoxigenin-3-[2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranosyl] (**20R 6b** and **20S 7b**). To a suspension of pre-hydrogenated 5% Pd-$CaCO_3$ in MeOH (40 ml) was added **2b** (2 g, 3.67 mmol) and stirred at room temperature for 24 hr, as previously reported.[3e] New prehydrogenated catalyst (800 mg) was added to the reaction mixture and stirred for another 24 hr. The mixture was filtered and the residue was washed with methanol and combined filterate was concentrated to dryness in vacuo. the crude product was crystallized from acetone-hexane to give 1.45 g (72%) of a mixture of **6b** and **7b**, as a powder, mp 186-188; $[\alpha]_D^{MeOH}$ = +5.90 (c=.305); IR(KBr) γ max 3500 (OH), 1770 (c=O) cm^{-1}; Anal ($C_{32}H_{50}O_7$) C,H. A 3.75×10^{-3} M solution of the mixture of **6b** and **7b** did not show any UV absorption at 217 nm, thus showing the absence of any unreacted 20(22)-ene starting material. The two diastereomers were separated cleanly by microscope slide TLC, using three developments (solvent System "A") as we have reported[15] for separation of the diastereomeric genins. Rf values were .76 for 20R-**6b** and .82 for 20S-**7b**.

a) Separation of **6b** and **7b**. (A typical separation followed by TLC as described above, began with a larger amount of **2b** than above.) Acetonide **2b** (4.5 g) was hydrogenated with 2.4 g of preactivated 5% Pd-$CaCO_3$ in 100 ml of MeOH as above. The reaction mixture was filtered by suction. The residue was washed with hot MeOH (130 ml X 3). After cooling the MeOH solution at room temp., 2.0 g of "I" was obtained by filtration. Recrystallization from MeOH four times gave 1.18 g of **6b** (mp 205-212°). The mother liquor separated from "I" was concentrated to dryness (1.3 g), and then recrystallized from EtOAc twice, giving crystals (763 mg, "II") enriched in **7b**. The filtrate from the original reaction mixture was evaporated in vacuo to afford 1.0 g of residue "III",and recrystallized from EtOAc twice to yield 448 mg enriched in **7b**. To this 448 mg was added "II" and followed by two more recrystallizations from EtOAc to yield 925 mg of 20S-**7b** (mp 197-201).

Multiple recrystallization of the mother liquor from "I,II,III" from a combination of MeOH and EtOAc as above afforded 707 mg (mp 207-212°) of **6b** (total 1889 mg, 83.6% based on approximately 1:1 mixture of R and S in **22**) and 578 mg (mp 196.5-201°) of **7b** (total 1494 mg, 66.1%).

(20R)-20,22-Dihydrodigitoxigenin-3-(2,6-dideoxy-β-D-ribo-hexopyranoside) (**6a**). A solution of **6b** (383 mg) in MeOH 200 ml was stirred with four drops of 5% HCl at room temperature for 18.5 hr. Work up in a similar manner as that of **3b** gave a crude mixture (245 mg). This was separated by dry column chromatography using CH_2Cl_2-EtOAc (9:1 to 3:7). The first fraction gave 29 mg (7.6%) of the starting material **6b** (mp 208-213°). The oily product (68 mg) from the second fraction was

recrystallized from EtOAc to 6 mg (16.1%) of genin **6a** (mp 211-212°), which we have previously reported[3c]. The third fraction afforded a crude oil (196 mg) which was crystallized from EtOAc to 155 mg (43.7% based on recovered starting material) of **6a**, mp 220-221°.

(20S)-20,22-Dihydrodigitoxigenin-3-(2,6-dideoxy-β-D-ribo-hexopyranoside) (**7a**). Acetonide **7b** (246 mg) was hydrolyzed with three drops of 5% HCl in a same way as that of **6b** to give recovered starting material **7b** (13 mg, 5.3%, mp 197-199° from EtOAc); the corresponding and previously reported[3e] genin **7c** (41 mg, 24.3%, mp 213-214° from EtOAc); and oily **7a** (129 mg). The **7a** thus obtained was recrystallized with EtOAc to afford 85 mg (37.3%) of **7a**, mp 211-214°.

22-Methylene-(20S)-20,22-Dihydrodigitoxigenin-3-[2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranoside] (**8b**). (20S)-Dihydro compound **6b** (821 mg, 1.5 mmol) in anhydrous THF 15 ml was reacted with N-isopropylcyclohexylamine (1.8 ml, 1.74 g, 9.96 mmol) in anhydrous THF (9 ml) and 6.6 ml (9.9 mmol) of 1.5 M butyllithium in hexane followed by treating of the produced enolate with anhydrous CO_2 in a similar manner as that of **5b**. The isolated intermediate (933 mg), presumably the carboxylic acid, was heated with 10 ml of 37% formalin (10% MeOH) and 3 ml of diethylamine on a steam bath for 40 min. Work up the reaction mixture gave crude product (800 mg) which was purified by dry column chromatography using CH_2Cl_2-EtOAc (9:1 to 8:2). The oily product obtained was crystallized from EtOAc to give 424 mg (50.5%) of **8b**, mp 196-200°.

22-Methylene-(20R)-20,22-Dihydrodigitoxigenin-3-[2,6-dideoxy-3,4-O-(1-methylethylidine)-β-D-ribo-hexopyranoside] (**9b**). The 22-methylene compound **9b** was synthesized from **7b** (600 mg, 1.1 mmol), N-isopropyl-cyclohexylamine (1.3 ml, 1.26 g, 7.23 mmole) in anhydrous THF (6.5 ml), 1.5 M butyllithium in hexane (48 ml, 7.19 mmol), 37% formalin (4 ml) and diethylamine (1.1 ml) by the same method as described above. The crude oil (660 mg) thus prepared was purified by dry column chromatography as above to give a pure oil, crystallized from ether-hexane to give 370 mg (60.4%) of **9b**, mp 179-182°.

22-Methylene-(20S)-20,22-dihydroditoxigenin-3-(2,6-dideoxy-β-D-ribohexopyranoside) (**8a**). Hydrolysis of **8b** (300 mg) in 200 ml of MeOH with 4 drops of 5% HCl for 12 hr produced 218 mg of crude oil in the same manner as that of **4b**. The oily product was separated by dry column chromatography using CH_2Cl_2-EtOAc (8:2 to 6:4) to afford 40 mg (13.3%) of starting material **8b** (mp 195-199°), 32 mg (15.3%) of the corresponding and previously reported[3e] genin **8c** (mp 234-240°) and a crude powder of **8a**. This was recrystallized from EtOAc-MeOH-CH_2Cl_2 to give 116 mg (41.7%) of **8a**, mp 204-205° (dec.). The analytical sample of **8a** was obtained by recrystallization from MeOH, giving 102 mg (36.7%) mp 223-228° (dec.).

22-Methylene-(20R)-20,22-Dihydrodigitoxigenin-3-(2,6-dideoxy-β-D-ribohexopyranoside) (**9a**). The (20R) diastereomer **9a** (153 mg, 51.5%, mp 163-164° (decomp.) was prepared by hydrolysis of 320 mg of **9b** in the same condition as that used for **8a**, together with 27 mg (8.4%) of unreacted material **9b**, mp 179.5-181.5° and 63 mg (28.3%) of genin **9c**, mp 127-132° (powder). The analytical sample of **9a** was obtained by recrystallization from AcOEt: yield 103 mg (34.6%), mp 193-194° (dec).

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