itors that have been studied clinically.

Inhibition of bovine lens aldose reductase was determined following the method of Hayman and Kinoshita[20] with DL-glyceraldehyde as substrate. The following IC_{50}'s were found: tolrestat, 3.5×10^{-8} M; alrestatin, 2.7×10^{-6} M; sorbinil, 1.5×10^{-6} M.

The doses that decreased galactitol accumulation by 50% in the sciatic nerve in the galactosemic rat model[4] after administration for 4 days in the diet were as follows: tolrestat, 7.3 ± 2.3 mg/(kg day); alrestatin, ~900 mg/(kg day).

In rats rendered diabetic with streptozotocin,[21] the doses that decreased sorbitol accumulation in the sciatic nerve by 50% after 3 weeks administration in the diet were as follows: tolrestat, 4.8 ± 2.4 mg/(kg day); alrestatin, ~1000 mg/(kg day).

Aldose reductase is present in the red blood cell (RBC), and sorbitol has been shown to accumulate in the human RBC when incubated in a high glucose-containing medium. This accumulation is blocked by the presence of the aldose reductase inhibitor tetramethyleneglutaric acid.[21] It has been suggested[21] that RBC sorbitol may be a useful indicator of the tissue sorbitol levels that participate in the pathogenesis of diabetes-associated complications. In rats rendered diabetic with streptozotocin, RBC sorbitol levels were 126 nmol/g of Hb, markedly higher than that seen in normal rats ~50 nmol/g of Hb). Treatment of diabetic rats with tolrestat in the diet for 7 days at a dose of 1.8 mg/(kg day) caused a reversal to normal RBC sorbitol levels.

On the basis of these and other pharmacological data to be published separately, tolrestat has been selected for clinical development and is currently being examined for the treatment of diabetic neuropathy.

Registry No. 1, 82964-04-3; 1 (methyl ester), 84533-04-0; 2, 84532-72-9; 2 (carboxamide derivative), 84533-46-0; 3, 61109-48-6; 4, 84532-68-3; 6, 85674-78-8; 7, 88245-14-1; 8, 88245-15-2; methyl sarcosinate, 5473-12-1; trifluoromethyl iodide, 2314-97-8; aldose reductase, 9028-31-3.

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Articles

Cardiac Glycosides. 1.[1] A Systematic Study of Digitoxigenin D-Glycosides

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A series of digitoxigenin glycosides was studied: five with β-D-sugars varying stepwise in sugar structure from β-D-digitoxose to β-D-galactose, including one β-D/α-D pair. I_{50} values for these glycosides and digitoxigenin were determined with hog kidney Na^+,K^+-ATPase. These data suggest a major and unexpected role for 4′-OH conformation in the sugar. All the glycosides with an equatorial 4′-OH were more active than the two with the 4′-OH axial [digitoxigenin β-D-galactoside (**6**) $I_{50} = 6.45 \times 10^{-8}$ M; digitoxigenin 2′-deoxy-α-D-*ribo*-hexopyranoside (α-**3a**) $I_{50} = 9.33 \times 10^{-8}$ M; digitoxigenin $I_{50} = 1.17 \times 10^{-7}$ M]. Stereochemistry of the 3′-OH had much less of an activity role than that of the 4′-OH, in contrast to existing models of "sugar-site" binding.

The biological roles of the sugars of natural and semisynthetic cardenolide glycosides have long been of considerable interest. In their classic reviews of 1962 and 1966, Zorbach and Reichstein[2] summarized the syntheses and LD50 data of a variety of sugar analogues of digitoxigenin (**1**) and strophanthidin. The extensive Na^+,K^+-ATPase studies by Yoda and Yoda[3] focused on a variety of naturally occurring monoglycosides (convallatoxin, helveticoside, ouabain, and deglucocheirotoxin), as well as on a variety of semisynthetic cardiac glycosides [digitoxigenin β-D-digitoxoside (**2**), digitoxigenin bisdigitoxoside, and digitoxin acetates]. Thomas, Brown, and co-workers[4] have reported the syntheses and guinea pig inotropic activities of digitoxigenin β-D-glucoside (**5**), digitoxigenin β-D-galactoside (**6**), digitoxigenin α-L-rhamnoside, and the glucoside and galactoside of digoxigenin.

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(1) Paper 2 in this series: Kihara, M.; Yoshioka, K.; Kitatsuji, E.; Deffo, T.; Fullerton, D. S.; Rohrer, D. C. *Steroids*, in press.
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(4) (a) Brown, L.; Boutagy, J.; Thomas, R. *Arzneim.-Forsch.* **1981**, *31*, 1059–1064. (b) Smith, P.; Brown, L.; Boutagy, J.; Thomas, R. *J. Med. Chem.* **1982**, *25*, 1222–1226.

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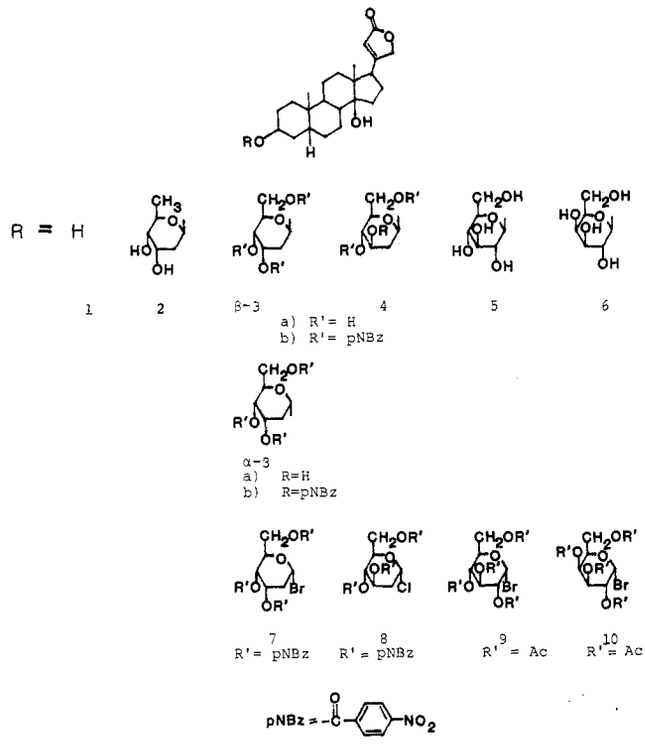
Chart I



As we will discuss, the array of genins, sugars, and biological systems studied has led to a variety of sometimes conflicting models on the role of sugar structure in biological activity. We have been very interested in resolving these models, and delineating roles of sugar conformation as well. We have used a combination of syntheses, conformational energy studies, MMS-X and PROPHET molecular graphics, and biological studies to delineate the structural and conformational roles of the C17 side group,[5] and we wanted to apply these same methods to the study of cardiac glycoside sugars.

Our work on cardiac glycosides began with the novel β-D-glucoside Actodigin of Ayerst—a very interesting glycoside whose genin has a "rotated" lactone ring.[6] Most recently, we have completed the syntheses of a wide variety of β-D-glucosides and galactosides, as well as β-D-digitoxosides, with widely varying C17 side groups.[1,8]

We report here the study of a series of digitoxigenin 3β-glycosides. These glycosides (Chart I) were systematically selected so that each sugar differs in a single structural feature as the series progresses from digitoxigenin β-D-digitoxoside [(3β,5β)-3-[(2',6'-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide, **2**] to digitoxigenin β-D-galactoside [(3β,5β)-3-(β-D-galactopyranosyloxy)-14-hydroxycard-20(22)-enolide, **6**]. Addition of a 6'-OH to **2** gives digitoxigenin [(3β,5β)-3-[(2'-deoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide, β-**3a**]. Inversion of the 3'-OH of **3a** gives 2-deoxyglucoside **4a** [(3β,5β)-3-[(2'-deoxy-β-D-*arabino*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide]. Addition of a 2'-OH to **4a** gives digitoxigenin β-D-glucoside [(3β,5β)-3-(β-D-glucopyranosyloxy)-14-hydroxy-card-20-(22)-enolide] (**5**). Finally, inversion of the 4'-OH of glucoside **5** gives digitoxigenin β-D-galactoside (**6**).

Results and Discussion

Chemistry. Digitoxigenin β-D-digitoxoside (**2**) was synthesized by the stepwise degradation of the two terminal digitoxoses of digitoxin as described by Satoh and Aoyama,[7] a procedure we also used in our previous study of Actodigin[6] and of modified digitoxosides.[8] Digitoxigenin β-D-glucoside (**5**) and β-D-galactoside (**6**) were synthesized from tetra-*O*-acetylglucosyl bromide (**9**) and tetra-*O*-acetylgalactosyl bromide (**10**) by a modified Koenigs–Knorr reaction.[1,11,13] The fact that the Cl' proton peaks in the ^{1}H NMR spectra are doublets with a 7-Hz coupling constant shows that glucosides **5a** and **6a** are the β-anomers,[8,9,4a] as one would predict from the usual course of these reactions.[10] (Syntheses and stereochemical assignments of **2**, **5**, and **6** are discussed in the accompanying papers in this series.[1,8])

The "6'-hydroxydigitoxoside" **4a** (IUPAC nomenclature has been given above) and the 2'-deoxyglucoside β-**3a** were synthesized by the methods of Zorbach and co-workers.[11,12] The needed tris-*O*-(*p*-nitrobenzoyl)-protected sugar halides **7** and **8** were obtained from 2'-deoxyglucose (**11**) and methyl glucoside (**13**) as shown in Scheme I.

The attempted condensation of digitoxigenin (**1**) with bromide **7** would not give the tris-*O*-(*p*-nitrobenzoyl) β-**3b** in the presence of silver carbonate in benzene,[13] nor silver salicylate,[8] nor Fetizon's reagent[4a] as possible acid-accepting reagents. However, we found that β-**3b** could be synthesized from **1** in a combination of ethylene dichloride and carbon tetrachloride with silver carbonate. Both β-anomer β-**3b** (35.8%) and α-anomer α-**3b** (crude yield 33.5%) were obtained, although Zorbach and Buhler have reported only the β-anomer using a similar reaction.[12]

Hydrolysis of β-**3b** and α-**3b** with methanolic aqueous potassium bicarbonate gave glycosides β-**3a** and α-**3a**. The X-ray crystal structure of these two anomers will be completed by us in the near future. However, a strong argument can be made for the assigned stereochemistry on the basis of the usual course of these reactions,[10] as well as

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(12) Zorbach, W. W.; Buhler, W. *Liebigs Ann. Chem.* **1973**, *670*, 116–121.

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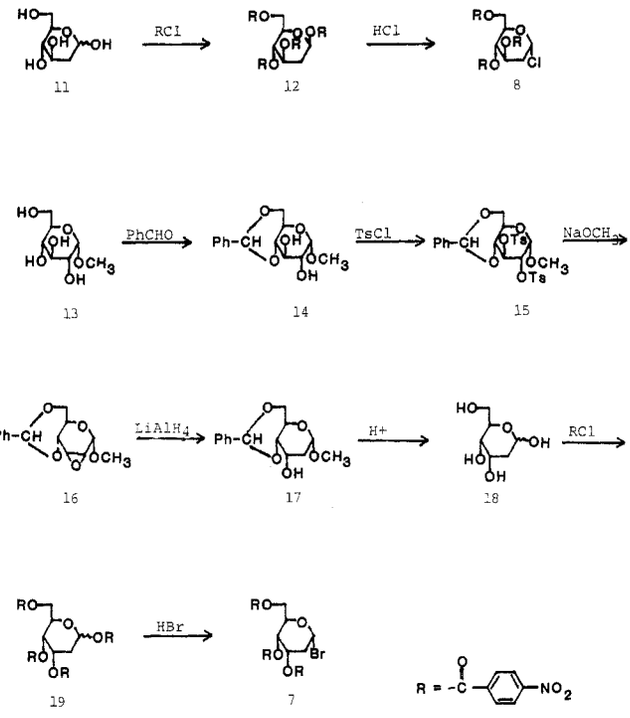
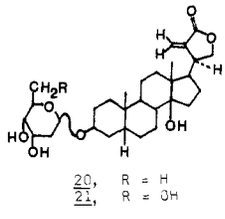
Scheme I



optical rotations. Klyne[14] found, for example, that β-anomers of D-sugar cardiac glycosides have more negative molecular rotations $[M]_D$ than that of the corresponding α-anomers. Klyne's "rule" was supported in other cardiac glycosides by Zorbach et al.[11] These data suggest strongly that the glycoside **3a** having a molecular rotation ($[M]_D$) of −22.4 ($[\alpha]_D$ −4.3°), is the β-anomer and that the glycoside **2a**, having a $[M]_D$ of +437.6 ($[\alpha]_D$ +84°), is the α-anomer. Furthermore, the configuration at Cl′ in β-**3a** is supported by the fact that its molecular rotation is very similar to that of the corresponding β-D-digitoxoside **2** ($[M]_D$ −28.2°).[8] Thus, changing the C6′ hydrogen to a hydroxy group (digitoxoside to "6′-hydroxydigitoxoside") also does not affect the molecular rotation. (We have found the same to be true with β-D-digitoxoside **20**[8] and 6-hydroxy-β-D-digitoxoside **21**[15]).



20, R = H
21, R = OH

The reaction of chloride 8 with digitoxigenin (**1**) and silver carbonate as described for **3a** above produced only the β-anomer **4b** (59.8%) without any α-anomer. Hydrolysis of **4b** gave glycoside **4a** (56.3%). The configuration of Cl′ in β-anomer **4a** was confirmed in the same way as for β-**3a**. For the β-anomer, the molecular rotation, $[M]_D$, was −36.9, whereas for α-anomer, the $[M]_D$ was +315.6.

Biology. Hog kidney Na^+,K^+-ATPase (E.C. 3.6.1.3) was used to determine the inhibitory activity of the glycosides and digitoxigenin. The enzyme was purified as described previously.[5b] The inhibition of the Na^+,K^+-ATPase was measured under equilibrium binding conditions employing type I binding conditions (i.e., with Mg^{2+}, Na^+, and ATP as the binding ligands). The enzyme was reacted with digitoxigenin in the presence of Mg^{2+}, Na^+, and ATP for a period of 10 min as described previously.[5b] This results in maximum binding of the drug to the Na^+,K^+-ATPase, as evidenced by maximal inhibition after this period of preincubation. With the glycosides, however, a 2-h preincubation was required to achieve maximal inhibition of the Na^+,K^+-ATPase under similar experimental conditions. This was established by incubating various glycosides in the above-described assay system for different periods of time and then determining the inhibition of Na^+,K^+-ATPase. Similar observations were made when equilibrium binding of [^{3}H]ouabain or a ^{3}H-labeled 24-azido photoaffinity probe analogue[18] to the enzyme was determined. After the initial preincubation (10 min for digitoxigenin; 2 h for glycosides), an appropriate amount of KCl was added (final concentration 10 mM) to initiate the Na^+,K^+-ATPase reaction. The reaction was terminated by adding cold trichloroacetic acid (10% w/v). Under these conditions, the Na^+,K^+-ATPase reaction proceeds at linear rates in the presence or absence of the drug. Na^+,K^+-ATPase in the presence or absence of added steroid was calculated as the activity in the presence of Mg^{2+}, Na^+, and K^+ minus that in the presence of Mg^{2+} and Na^+. The very small basal Mg^{2+} plus Na^+ dependent ATPase activity in these preparations was not affected by the steroids. (All the steroids were added as ethanolic solutions. The maximum ethanol added per 2-mL reaction was 20 μL; this amount has no significant effect on the ATPase. Controls included ethanol at this same concentration.) All other details of the assay have been given previously.[5b]

I_{50} values (concentration required for 50% inhibition of the Na^+,K^+-ATPase) were calculated as described previously.[5d,g] These results are shown with the sugar conformational diagrams in Table I. Each determination was confirmed at least three times; the result did not vary by more than ±5%.

The glycoside sugars selected for study were chosen on the basis of their varied and systematic pattern of hydroxyl position. These structural variations include axial vs. equatorial 3′-OH and 4′-OH, the presence or absence of the 6′-OH, the presence or absence of the 2′-OH, and blockade of the 3′- and 4′-hydroxyl groups. Previous studies by Yoda and Yoda[3] have shown that under type I binding conditions, the genin portion of the glycosides is primarily responsible for controlling the association rate for binding to Na^+,K^+-ATPase, while both the nature of the genin and the sugar moieties influence the dissociation characteristics of the cardiac glycoside.[3d] These changes in rate directly affect the I_{50} for the glycosides. In these studies, digitoxigenin (**1**) was the only genin used as the steroid portion of the glycosides. This eliminated any potential source of I_{50} variation resulting from changes in the genin moiety on either the association or dissociation rates. Thus, the equilibrium binding I_{50} values obtained from these comparative studies primarily reflect variations in the sugars' contribution to the dissociation rate of the glycosides.

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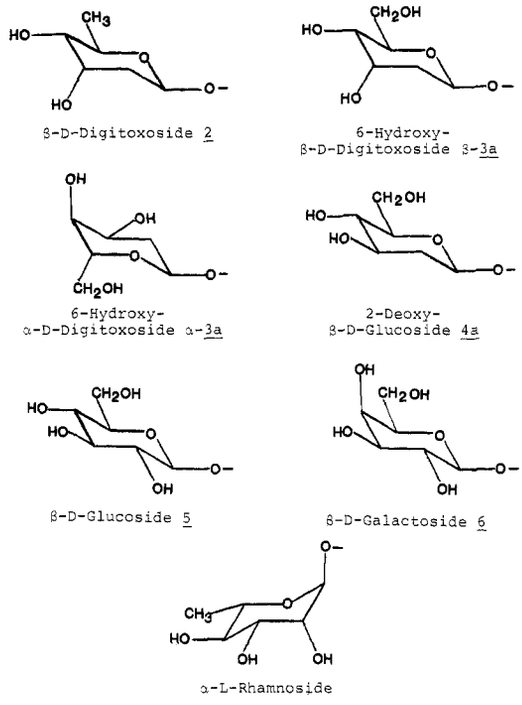
Chart II. Conformations of Glycoside Sugars



The Na^+,K^+-ATPase "sugar-site" binding model proposed by Yoda and co-workers emphasizes the importance of stereochemistry of the sugar 3′-OH.[3] In their view, if the 3′-OH is axial, binding of the sugar to the Na^+,K^+-ATPase would be expected to be much better than if this OH is equatorial. Consistant with the model, the least active of the glycosides tested were **6** and **α-3a**, and both have the 4′-OH axial and the 3′-OH equatorial (Chart II). Similarly, the analogues with the 4′-OH equatorial and 3′-OH axial, **2** and **β-3a**, were both found to be very active Na^+,K^+-ATPase inhibitors.

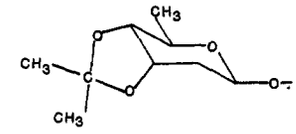
The major difference between the results reported here and those of Yoda is that changes in the equatorial vs. axial positioning of the 4′-OH correlate with the activity in the hog kidney Na^+,K^+-ATPase system far better than similar changes of the 3′-OH positioning. The analogues with 4′-OH equatorial and 3′-OH equatorial, **4a** and **5**, were found to be very active, in direct conflict with the model (which would predict a lower activity for these analogues due to the equatorial positioning of the 3′-OH). Thus, in each case (Chart II) the sugar moiety with an equatorial 4′-OH substituent was found to be very active, while the corresponding 4′-OH axial analogues were less active. This result was relatively independent of 3′-OH positioning. (After this paper was submitted, Brown and Thomas published a report that also suggests an important role for the sugar 4′-OH.[21])

The enhancement of activity by an equatorial 4′-OH also may explain the observations of Stache and co-workers[19] that the 2,3-dideoxy-α-L-rhamnoside (which has neither a C2′-OH nor C3′-OH) of digitoxigenin is as active as digoxin. (It is important to note, however, that experiments with Na^+,K^+-ATPase were not reported.) It does have an equatorial 4′-OH, which could account for the glycoside's high activity. Furthermore, as we have noted before,[5a] ouabain—the most widely used glycoside in studying Na^+,K^+-ATPase—may not fit the proposed sugar-site binding model. The conformation of α-L-rhamnose both as the free sugar and in ouabain has the 3′-OH equatorial.[20] Nevertheless, this sugar is a substantial potentiator of genin activity.[3] (The α-L-rhamnose drawing used in formulation of the Yoda model had the 3′-OH axial.[3]) However, the two observed crystallographic conformations for ouabain's α-L-rhamnoside also have an equatorial 4′-OH,[20] consistent with our observations that an equatorial position for the 4′-OH enhances activity.

Table I. Hog Kidney Na^+,K^+-Dependent ATPase Inhibition

steroid	I_{50}, M
1	1.17×10^{-7}
2	7.04×10^{-9}
2 acetonide	4.18×10^{-8}
β-3a	1.07×10^{-8}
α-3a	9.33×10^{-8}
4a	2.82×10^{-8}
5	1.17×10^{-8}
6	6.45×10^{-8}

This view is supported by the activity of the 3′,4′-acetonide of **2**. This sugar derivative has both the 3′-OH



2 acetonide

and 4′-OH blocked but shows considerable potentiation of genin activity (Table I)—better, for example, than digitoxigenin galactoside **6**, which does have an axial 3′-OH. This finding contrasts with the observations of Yoda, wherein acetylation of the 3′-OH of **2** markedly decreases activity.[3]

The presence of an equatorial 2′-OH on the sugar moiety does seem to enhance activity, as can be seen in the increase in the activity of **5** over the corresponding sugar **4a** with no 2′-OH. Similarly, the presence of a hydroxyl group on C6′ seems to cause a decrease in activity from that of the corresponding 6′-deoxy sugar (see the activity for analogues **β-3a** vs. **2**). Both of these observations are also consistent with the structural model proposed by Yoda.[3] However, their effects are smaller than that observed from optimal positioning of the 4′-OH.

Still, other factors may affect binding to the sugar binding site on Na^+,K^+-ATPase. The data reported here were obtained with hog kidney Na^+,K^+-ATPase, while Yoda's work was based on Na^+,K^+-ATPase from beef brain. Our earlier studies using rat brain Na^+,K^+-ATPase[6] showed that addition of a β-D-glucose to digitoxigenin had little effect on activity, yet the present studies show nearly a tenfold increase in activity with hog kidney enzyme. This indicates that there may be differences in the "sugar binding site", which are dependent on the source of the enzyme used. We are now investigating these glycosides in a variety of Na^+,K^+-ATPase systems. In addition, we are also looking at additional variations in the sugar (including L sugars) and genins with different types of C17 side groups.

The orientation of the sugar relative to the genin may also play a role in determining the binding ability of the

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(21) Brown, L.; Thomas, R. *Arzneim.-Forsch.* **1983**, *33*, 814–817.

sugar. We have recently reported the first of our sugar conformational energy studies—including the surprising finding that the sugar attached to the genin C3 has very little conformational freedom about the Cl′–O–C3 bonds.[5d] Preliminary structural results from crystallographic studies have shown a similar result. While the orientation of the sugar about the C3–O bond shows variations of 99°, the orientations about the O–Cl′ bond appear to be surprisingly limited with variations of only 33°. These results, however, should not detract from the observation concerning the role of the 4′-OH in enhancing the activity of cardiac glycosides.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW laboratories, Phoenix, AZ. The 100-MHz ^{1}H NMR spectra were taken at the Oregon State University NMR Spectroscopy Laboratory, Department of Chemistry. IR spectra were run as KBr pellets on a Beckmann Model Acculab 7 spectrophotometer. Optical rotations in methanol were taken on a Perkin-Elmer 141 polarimeter. Thin-layer chromatography was performed on 0.25-mm EM silica gel 60 F-254 glass plates by techniques and solvent combinations as we have previously described.[16] "Flash chromatography" following the method of Still and co-workers[17] used silica gel 60, 230–400 mesh (EM Merck), in a column 4 cm in diameter and 20 cm high, with a combination of CH_2Cl_2 and AcOEt (10:1) as solvent.

We have previously reported[6] the synthesis of glucoside **5** following the modified Koenigs–Knorr procedure of Takiura and co-workers[13] (silver carbonate in benzene with azeotropic removal of water). It was reported much earlier by Zorbach and Reichstein[2] and more recently by Thomas and co-workers.[4] The synthesis of galactoside **6** and a variety of other glucosides and galactosides following the Koenigs–Knorr modification of Wolff and co-workers (silver salicylate, with no desicating procedure being necessary) was recently reported.[1] The synthesis of digitoxoside **2** and its acetonide followed the digitoxin stepwise degradation procedure of Satoh and Aoyama,[7] as we have described earlier.[6] A variety of other digitoxosides with modified C17 side groups will be reported in the near future.[8]

(3β,5β)-3-[(3′,4′,6′-Tris-*O*-(*p*-nitrobenzoyl)-2′-deoxy-β-D-*arabino*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (4b). A mixture of digitoxigenin (1; 374 mg, 1 mmol), silver carbonate (1.0 g, 3.62 mmol), and anhydrous ethylene dichloride (70 mL) was heated with stirring, shielded from light, and protected from atmospheric moisture with a drying tube. After 30 mL of the solvent was distilled, a solution of 8 (1.52 g, 2.4 mmol) in anhydrous ethylene dichloride (70 mL) and carbon tetrachloride (70 mL) was added dropwise to the mixture over 3.75 h while the distillation rate was regulated so as to keep the volume of the reaction mixture constant. At the end of that 3.75-h, period, ethylene dichloride (96 mL) was added over 2.5 h, followed by carbon tetrachloride (50 mL), again keeping the reaction volume nearly constant. The reaction mixture was cooled to room temperature and filtered by suction, and the residue was washed with CH_2Cl_2. The filtrate was evaporated in vacuo to give an amorphous powder (2.0 g), which was subjected to flash chromatography. The first fraction gave 579 mg (59.8%) of **4b** as an amorphous powder, mp 150–155 °C. (This was hydrolyzed without further purification to **4a**): IR (KBr) 3440 (broad, OH), 1775 (shoulder) and 1720 (broad) (C=O), 1620 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 8.36–7.96 (12 H, m, with major peaks at 8.22, 8.20, 8.14, and 8.09, aromatic H), 5.87 (1 H, m, $W_{h/2}$ = 3 Hz, C_{22} H), 5.66–3.94 (6 H, m, with major peaks at 5.58, 5.49, 4.61, 4.58, and 4.15, $C_{1'}$ and $C_{3'}$–$C_{6'}$ H), 4.89 (2 H, qd, J = 18 and 1.5 Hz, C_{21} H), 4.08 (1 H, m, C_3 H), 0.93 (3 H, s, C_{19} H), 0.89 (3 H, s, C_{18} H).

(3β,5β)-3-[(3′,4′,6′-Tris-*O*-(*p*-nitrobenzoyl)-2′-deoxy-α- and -β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolides (α- and β-3b). The reaction of digitoxigenin (1; 374 mg, 1 mmol) in anhydrous ethylene dichloride (50 mL) with silver carbonate (800 mg, 2.9 mmol) and bromide **7** (1.35 g, 2 mmol) in anhydrous ethylene dichloride (50 mL) and carbon tetrachloride (60 mL) was carried out in a same manner as described above for the synthesis of **4b**. After the solution of **7** was added to the mixture, 100 mL of ethylene dichloride was added over 1 h with simultaneous distillation to keep the volume constant. The mixture was then stirred at room temperature for an additional 0.5 h. The reaction mixture was filtered, and the residue was washed with acetone (30 mL × 3). The filtrate was evaporated to an oil, which was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$, and evaporated to an amorphous powder. This crude product was purified by flash chromatography.[17] The first fraction gave 344 mg of crude α-**3b**, which was hydrolyzed without any further purification (as described later). The integral values in the ^{1}H NMR spectrum of crude α-**3b** showed that it contained a sugar: ^{1}H NMR ($CDCl_3$) δ 8.42–7.98 (m, with major peaks at 8.26, 8.18, 8.11, and 8.08, aromatic H), 5.88 (m, $W_{h/2}$ = 4 Hz, C_{22} H), 6.02–4.46 (m, with major peaks at 5.81, 5.52, 5.42, and 4.74, $C_{1'}$, $C_{3'}$, and $C_{6'}$ H), 4.06 (m, C_3 H), 0.88 (s, C_{19} H), 0.76 (s, C_{18} H); IR (KBr) 3440 (broad, OH), 1725 (C=O) cm^{-1}.

The second fraction afforded 434 mg of crude β-anomer β-**3b**. The product was crystallized from CH_2Cl_2–hexane to give 302 mg (35.8% based on reacted 1) of β-**3b**: mp 162–164 °C; IR (KBr) 3420 (broad, OH), 1725 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42–7.96 (12 H, m, with major peaks at 8.27, 8.24, 8.26, 8.19, 8.15, and 8.06, aromatic H), 5.87 (1 H, m, $W_{h/2}$ = 4 Hz, C_{22} H), 6.04–5.03 (3 H, m, with major peaks at 5.95, 5.50, 5.42, 5.39, and 5.21, $C_{3'}$–$C_{5'}$ H, 4.89 (2 H, qd, J = 18 and 1.5 Hz, C_{21} H), 4.73–4.42 (3 H, m, with major peaks at 4.63, 4.58, 4.55, and 4.48, $C_{1'}$ and $C_{6'}$ H), 4.10 (1 H, m, C_3 H), 2.80 (1 H, m, C_{17} H), 0.94 (3 H, s, C_{19} H), 0.88 (3 H, s, C_{18} H). Anal. ($C_{50}H_{53}O_{17}N_3$) C, H, N.

The third fraction gave an oil (179 mg), which was recrystallized from CH_2Cl_2–hexane to 82 mg of recovered 1, mp 235–240 °C.

(3β,5β)-3-[(2′-Deoxy-β-D-*arabino*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (4a). To 400 mg of tris-*O*-(*p*-nitrobenzoyl) derivative **4b** in 200 mL of MeOH was added a solution of $KHCO_3$ (2.5 g) in water (90 mL). The mixture was stirred at room temperature for 12 days, with the disappearance of **4b** followed on TLC using CH_2Cl_2–AcOEt–MeOH (20:2:7). The reaction mixture was concentrated at 30 °C to 90 mL and extracted with CH_2Cl_2 four times. The extracts were washed with water, dried over $MgSO_4$, and evaporated to a white powder. Recrystallization of the product from MeOH–AcOEt gave white needles of **4a**: mp 225–229 °C; $[\alpha]^{22}_D$ −8.9° (*c* 0.235, MeOH); yield 56.3% (overall yield 33.7% from genin 1) [lit.[8] mp 226–230 °C; $[\alpha]^{25}_D$ +11.8° (*c* 1.21, MeOH)]; IR (KBr) 3400 (OH), 1730 (broad, C=O), 1625 (C=C) cm^{-1}; ^{1}H NMR (25% Me_2SO-d_6 in $CDCl_3$) δ 5.82 (1 H, m, $W_{h/2}$ = 4 Hz, C_{22} H), 4.89 (2 H, qd, J = 18 and 1.5 Hz, C_{21} H), 4.73–4.10 (3 H, m, with major peaks at 4.57, 4.55, 4.50, and 4.48, $C_{1'}$, $C_{3'}$, and $C_{4'}$ H), 4.02 (1 H, m, C_3 H), 0.91 (3 H, s, C_{19} H), 0.84 (3 H, s, C_{18} H). Anal. ($C_{29}H_{44}O_8 \cdot H_2O$) C, H.

(3β,5β)-3-[(2′-Deoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (β-3a). To a suspension of 400 mg of the tris-*O*-(*p*-nitrobenzoyl) derivative β-**3b** in MeOH (250 mL) was added a solution of $KHCO_3$ (2.5 g) in water (90 mL) and stirred for 12 days at room temperature. The reaction mixture was treated in a similar manner as **4a** to give white crystals (249 mg). This crude product was recrystallized from EtOH to afford 165 mg (76.7%) of β-**3a**: mp 223–225 °C; $[\alpha]^{22}_D$ −4.3° (*c* 0.325, MeOH) [lit.[9] mp 223–225 °C; $[\alpha]^{25}_D$ +27° (*c* 0.63, EtOH)]; IR (KBr) 3400 (broad, OH), 1725 (C=O) cm^{-1}; ^{1}H NMR (25% Me_2SO-d_6 in $CDCl_3$) δ 5.84 (1 H, m, $W_{h/2}$ = 3 Hz, C_{22} H), 4.95 (2 H, qd, J = 18 and 1.5 Hz, C_{21} H), 5.01–3.40 (6 H, m, with major peaks at 4.35, 4.29, 4.03, 3.72, 3.66, and 3.59, $C_{1'}$, $C_{3'}$, and $C_{6'}$ H), 4.03 (1 H, m, C_3 H), 0.91 (3 H, s, C_{19} H), 0.86 (3 H, s, C_{18} H). Anal. ($C_{29}H_{44}O_8$) C, H.

(3β,5β)-3-[(2′-Deoxy-α-D-*ribo*-hexopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (α-3a). The crude tris-*O*-(*p*-nitrobenzoyl) derivative α-**3b** (300 mg) in MeOH (250 mL) was hydrolyzed with $KHCO_3$ (2.5 g) in water (90 mL) in the same way as for the synthesis of β-**3a**. Recrystallization of the hydrolyzed product (125 mg) from MeOH–AcOEt afforded 61 mg (15.7% from genin 1) of α-**3a**: mp 228–232 °C; $[\alpha]^{22}_D$ +84° (*c* 0.30, MeOH); IR (KBr) 3460 (broad, OH), 1740 (broad, C=O), 1625 (C=C) cm^{-1}; ^{1}H NMR (5% Me_2SO-d_6 in $CDCl_3$) δ 5.86 (1 H, m, $W_{h/2}$ = 3 Hz, C_{22} H), 4.92 (2 H, qd, J = 18 and 2 Hz, C_{21} H), 5.11–3.40 (6 H, m, with major peaks at 5.07, 5.04, 4.06, 3.84, 3.70, 3.56, and 3.48, $C_{1'}$, $C_{3'}$, and $C_{6'}$ H) 4.02 (1 H, m, C_3 H), 0.95 (3 H, s, C_{19} H), 0.88 (3 H, s, C_{18} H). Anal. ($C_{29}H_{44}O_8$) C, H.

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A Colon-Specific Drug-Delivery System Based on Drug Glycosides and the Glycosidases of Colonic Bacteria[1]

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Steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a new colon-specific drug-delivery system. Drug glycosides are hydrophilic and, thus, poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa. This concept was illustrated with dexamethasone 21-β-D-glucoside (1) and prednisolone 21-β-D-glucoside (2), two prodrugs that may be useful in treating inflammatory bowel disease. Hydrolysis of the prodrugs by β-glucosidase and fecal homogenates in vitro released the free steroids. Glucosides 1 and 2 were administered to rats intragastrically to determine when and where the free steroids were released. Unmodified dexamethasone (3) and prednisolone (4) were also given to rats intragastrically to compare absorption of the glucosides with the free steroids. Both glucosides were found to reach the rat lower intestine in 4–5 h, where they were rapidly hydrolyzed, releasing the free steroids. Delivery of steroid 3 (via glucoside 1) was more specific than that of steroid 4 (via glucoside 2): nearly 60% of an oral dose of glucoside 1 reached the cecum, whereas less than 15% of glucoside 2 reached the cecum. When free steroids 3 and 4 were administered orally, they were almost exclusively absorbed in the small intestine: less than 1% of an oral dose of each reached the cecum.

The delivery or activation of drugs at specific sites to reduce side effects and increase pharmacological response has received increased emphasis recently. Implantable pumps,[2,3] adhesive patches impregnated with drugs,[3] vesicle-enclosed drugs,[4,5] and drug carriers[6] have all been proposed to achieve site-specific drug delivery. Prodrugs[7,8] have also been used in drug-delivery systems. A prodrug is inactive as administered, but its physicochemical properties permit its activation in vivo once it reaches its target. Prodrugs have been used to deliver drugs systemically to the kidneys,[9,10] brain,[11,12] breasts,[13] and central nervous system[14,15] and topically to the eyes[16] and skin.[17,18] Prodrugs are also currently being designed for use in cancer chemotherapy.[19–21] In all these cases, the prodrug is converted to the parent drug chemically or by specific enzyme(s) at the target site.

Colon-specific delivery of bioactive compounds is known to occur in man. In the plant kingdom, a great many compounds are found as glycosides.[22–32] Upon ingestion, many of these glycosides pass through the upper intestine and into the colon. Once there, the glycosidases of the colonic microflora liberate aglycons, which can then act on the colon. Certain sulfa drugs[33] are now known to be

(1) A preliminary report on this work has been presented; see: Abstracts, 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, American Societies for Experimental Biology: Bethesda, MD, 1983; abstr. 6298, Gastrointestinal Pharmacology section.
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