Cardiac Glycosides. 6. Gitoxigenin C16 Acetates, Formates, Methoxycarbonates, and Digitoxosides. Synthesis and Na^+,K^+-ATPase Inhibitory Activities

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A series of 17 gitoxigenin 16β-formates, acetates, and methoxycarbonates was synthesized, including their 3β-acetates, formates, and digitoxosides. A 16β-formate group was generally found to increase activity 30 times, a 16β-acetate group 9–12 times, while a 16β-methoxycarbonate decreased activity by two-thirds. 3β-Formates and acetates had little effect on activity by themselves, but sometimes reduced the activity-increasing properties of 16β-formates and acetates. A 3β-digitoxoside increases the activity of gitoxigenin by 15 times, but the effect is less if the 16β-group is esterified. And finally, a 16-one decreases activity dramatically. These data suggest an important role for C16 esters and possibly the presence of a separate binding site on Na^+,K^+-ATPase corresponding to the cardenolide C16 position.

Gitaloxigenin (**6**, gitoxigenin 16β-formate) and its tridigitoxoside gitaloxin are naturally occurring cardenolide 16β-formates found in *Digitalis purpurea*.[3] Interest in gitaloxin has recently increased, and it has even been proposed that this "forgotten cardiac glycoside of *Digitalis purpurea*" may be responsible for most of purpurea's therapeutic activity.[4a] In studies with a guinea pig heart Na^+,K^+-ATPase assay system, Depover and Godfraind found that gitaloxigenin (**6**) is 5 times more potent than digitoxigenin and 41 times more potent than gitoxigenin (**2**).[4b] We have been studying the relationship of cardenolide structure and Na^+,K^+-ATPase inhibitory activity and have found an excellent correlation with C17 side group carbonyl oxygen position obtained from crystallographic results.[1,5–8] Thus, we wished to extend these studies to examine in detail the effects of 16β-substitution on cardenolide activity.

In the present study, we report the synthesis of a series of gitoxigenin 16β-formates, acetates, methoxycarbonates, 16-ones, and their biological activity (inhibition of Na^+,K^+-ATPase). Since previous studies have also shown that the sugar directly attached to the genin has the greatest role in sugar site binding,[1,9,10] we also made the monodigitoxoside **24** (the monodigitoxoside analogue of gitaloxin) and its acetate analogue **22**. Preliminary accounts of the crystallographic and computer graphics analyses of some of these and related compounds have recently been published.[2] A detailed account of these investigations will be published separately in the near future.

Chemistry. The synthesis of acetates **3**, **4**, **5**, and β-D-digitoxoside **19** were modifications of methods previously reported by Satoh and co-workers.[11–14]

As shown in Table I, several experiments were conducted to delineate conditions for selective acetylation of the 3β- or 16β-hydroxyl groups. Selective acetylation of gitoxigenin **2** to make 16β-acetate **5** was best achieved at 17 °C with acetic anhydride and pyridine. Yields were typically 70%. Reaction for more than 1 h results in increasing amounts of the 3β,16β-diacetate **3**. In contrast, formic acetic anhydride in pyridine produces the 3β,16β-diformate **8** even at 0–5 °C. The desired 16β-formate **6** could be made instead with formic acetic anhydride and sodium formate in DMF, albeit not as selectively (45% of **6**, 13% of the 3β-formate **7**, and 19% of the 3β,16β-diformate **8**. Selective acetylation of **2** to produce 3β-acetate **4** (in 56.9% yield) was best with acetic anhydride in acetic acid and chloroform. Alternatively, the 3β,16β-diacetate **3** could be selectively hydrolyzed to **4** in 65% yield in methanolic potassium bicarbonate. These same methods were then used to produce the mixed esters **9** and **10**.

In all cases, the 3β-esters could be easily distinguished from the 16β-esters by chemical shifts of the C16 and C3 protons.[15] Proton NMR shifts were consistent with those previously reported by Tori and Aono.[15] A new and interesting finding is that the C15 protons are diasterotropic. In gitoxigenin 16β-acetate (**5**) and gitoxigenin 3β,16β-diacetate (**3**) the C15 protons appear at δ 2.75 (dd, J = 16, 10 Hz, 1 H, C15 H^1) and 1.82 (m, 1 H, AM part of an AMC system superimposed on the methylene protons of the rest of the steroid ring system, C15 H^2). These assignments were made with two-dimensional $^1H/^1H$ and $^{13}C/^1H$ spectra, both obtained on a Brucker 400-MHz instrument. The C15 H^1 proton at δ 2.75 is coupled to the C15 H^2 at δ 1.82. Both the C15 H^1 and H^2 protons are coupled to

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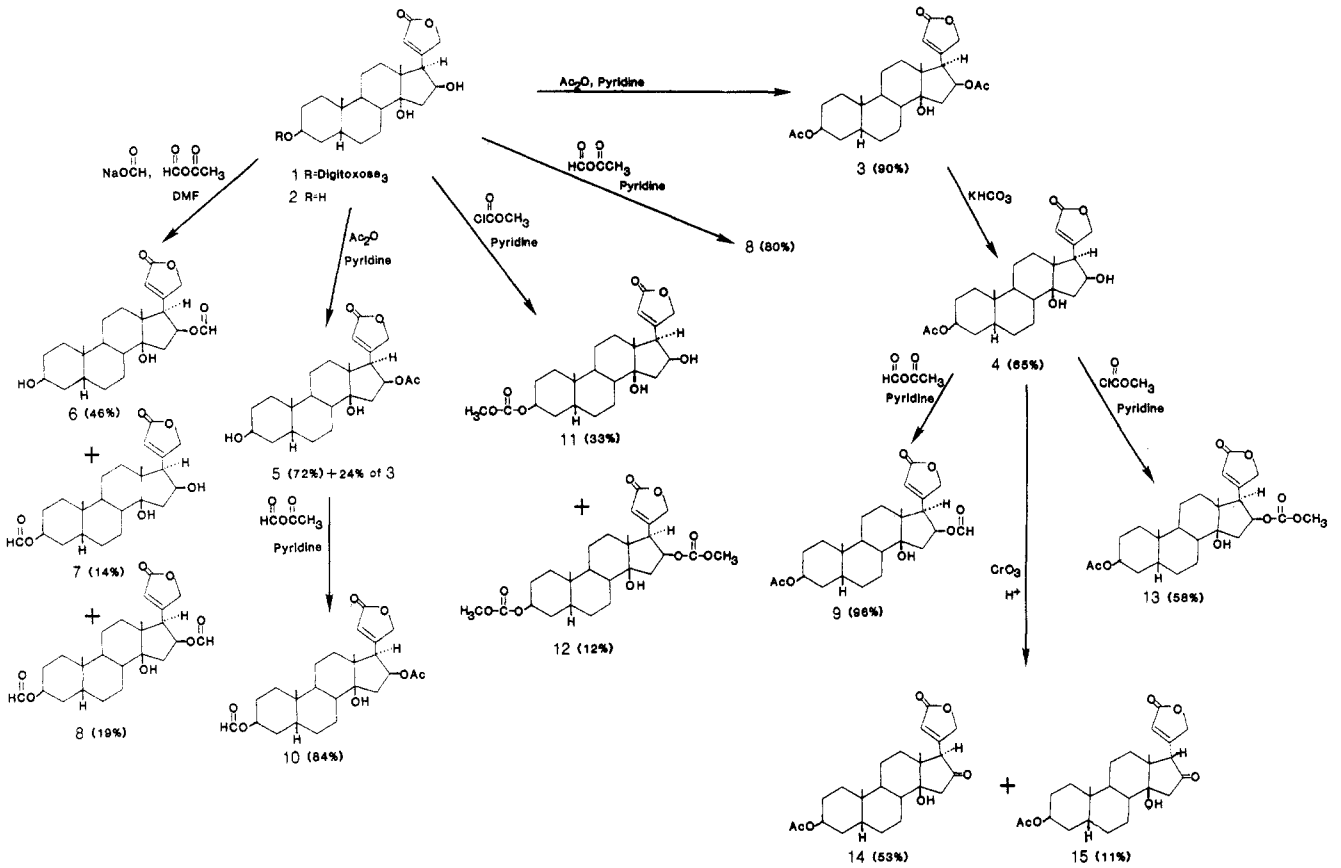
Scheme I



the C16 proton at δ 5.45. In the $^{13}C/^{1}H$ spectrum, two protons at δ 2.75 and 1.82 are coupled to C15 at 41.0 ppm.

To learn if the electronic character of the oxygen functionality at C16 was an important determinant of activity, we prepared the analogues series of methoxycarbonates, as well as the 16-one **14**. The 3β-methoxycarbonate **11** and 3β,16β-dimethoxycarbonate **12** were prepared from gitoxigenin (**2**), by treatment with methoxycarbonyl chloride in pyridine. The mixed ester **13** was similarly prepared from 3β-acetate **4** in 58% yield. Jones oxidation of **4** gave the 16-one analogue **14**, along with its 17α-isomer **15**.

Using a modification of the procedures we have previously used for synthesis of digitoxigenin 3β-digitoxoside,[8] we made the bisdigitoxoside **18** via dialdehyde **16** and diol **17** as shown in Scheme II. Repeat of the same series of steps (periodate oxidation, sodium borohydride reduction to the diol, and acid hydrolysis) gave monodigitoxoside **19** in 70% yield from **18**.

Protection of the 3′- and 4′-hydroxyls of **19** as the acetonide (using methods we have previously reported for other cardenolide digitoxose acetonides[8]), followed by esterification of the 16β-hydroxyl as described above for the genin analogues, and acid hydrolysis removal of the acetonide group[16] gave **22**, the 16β-acetate analogue of **19**, and **24**, the 16β-formate analogue of **19**.

Biology. As in our previous studies, a hog kidney Na^+,K^+-ATPase preparation was used for studying the activity of cardiac steroids.[5,6] In brief, the inhibition was measured under type I binding conditions (i.e., with Mg^{2+}, Na^+, and ATP as the binding ligands, 15-min preincubation for genins and 2 h for glycosides). All assays were carried out under equilibrium drug binding conditions and during the linear phase of the ATPase reaction.[5] The resulting I_{50} values (concentration required for 50% inhibition of the Na^+,K^+-ATPase) are shown in Table II. Each I_{50} was confirmed at least three times and the results did not vary by more than 5% in any case.

Effect of the 16β-OH. As can be seen in Table II, gitoxigenin is only 20% as active as digitoxigenin. This is consistent with the activity-decreasing effect of a 16β-OH reported by Depover and Godfraind.[4b]

Effect of a 16β-Formate. A 16β-formate increases genin activity about 30-fold. Examples include gitoxigenin 16β-formate (**6**) vs. gitoxigenin (**2**), 30 times increase; the corresponding 3β-acetates **9** vs. **4**, 35 times increase.

Effect of a 16β-Acetate. A 16β-acetate increases genin activity less than a 16β-formate, only 9–12-fold. Examples include gitoxigenin 16β-acetate (**5**) vs. gitoxigenin (**2**), 12 times increase, and the corresponding 3β,16β-diacetate **3** vs. 3β-acetate **4**, 8 times increase.

Effect of a 3β-Formate. A 3β-formate has little effect on activity and, in the presence of a 16β-ester, may decrease activity slightly. Examples include gitoxigenin (**2**) vs. 3β-formate **7**, no effect; 3β,16β-diformate **8** vs. 16β-formate **6**, no significant change; and 3β-formate 16β-acetate **10** vs. 16β-acetate **5**, slight decrease.

Effect of a 3β-Acetate. A 3β-acetate has little effect on activity and, in the presence of a 16β-ester, may decrease activity slightly. Examples include gitoxigenin (**2**) vs. 3β-acetate **4**, no effect; 3β,16β-diacetate **3** vs. 16β-acetate **5**, slight decrease; and 3β-acetate, 16β-formate **9** vs. 16β-formate **6**, no significant change.

Effect of a 16β-Methoxycarbonate. A 16β-methoxycarbonate decreases activity, as shown with **12** vs. gitoxigenin (**2**), loss of two-thirds of activity.

Effect of a 3β-Digitoxoside. With digitoxigenin analogues, as reported in the previous paper in this series, a 3β-digitoxoside increases activity by an average of 8.9 times. However, a different relationship exists with gitoxigenin analogues. If the 16β-OH is unsubstituted, the

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Table I. Acetylation and Formylation of Gitoxigenin

gitoxigenin (2)	reagents	temp, °C	time, h	products	recovered 2, %
1.0 g	Ac_2O (2 mL)	17	1	72% **5**	
	pyridine (10 mL)			24% **3**	
10.0 g	Ac_2O (50 mL)	17	14	90% **3**	
	pyridine (60 mL)				
700 mg	Ac_2O (70 mL)	60	50 min	50% **4**	48
				trace **5**	
1.0 g	Ac_2O (100 mL)	60	2	30% **4**	45
				16% **3**	
				trace **5**	
300 mg	Ac_2O (100 mL)	0–5	1	26% **4**	59
	H_2SO_4 (half drop)			3% **3**	
				5% **5**	
8.0 g	Ac_2O (160 mL)	60–70	2	57% **4**	
	AcOH (8 mL)			18% **3**	
	$CHCl_3$ (100 mL)				
300 mg	Ac_2O (60 mL)	0–5	1	26 **4**	59
	AcOH (60 mg)			5% **5**	
	$ZnCl_2$ (300 mg)			3% **3**	
1.0 g	$HCOOCOCH_3$ (5 mL)	0–5	1	80% **8**	
	pyridine (10 mL)				
3.0 g	$HCOOCOCH_3$ (23 mL)	17	1	14% **7**	
	HCOONa (300 mg)			46% **6**	
	DMF (70 mL)			14% **7**	
				19% **8**	
2.0 g	$ClCOOCH_3$ (6 mL)		6	33% **11**	27
	pyridine (30 mL)	0–5		12% **12**	

effect with gitoxigenin analogues is slightly larger—about a 15-fold increase, e.g., digitoxoside **19** vs. gitoxigenin (**2**). In contrast, the effect of a β-digitoxose is apparently less if the 16β group is esterified. Examples include 16β-formate **6** vs. its digitoxoside **24**, about a 7-fold increase; 16β-acetate **5** vs. its digitoxoside **22**, about a 7-fold increase; and gitoxin 16β-acetate (**26**) vs. gitoxigenin 16β-acetate (**5**), about a 5-fold increase.

These data thus show a consistent pattern of activity enhancement for small C16β-esters, particularly a C16β-formate. As other have also observed,[4] a C16β-OH decreases digitoxigenin's activity. In preliminary crystallographic and computer graphics studies, we found that the decreased activity of gitoxigenin can be explained by its C17 side group carbonyl oxygen position.[2] (The gitoxigenin 16-one analogues have not yet been studied in detail.) On the other hand, observations with gitoxigenin C16-esters[2] show that their altered C17 side group carbonyl oxygen positions do not explain their enhanced biological activities. An additional factor is clearly important. One possibility could be a separate C16 ester binding site on the receptor. Alternatively, as suggested by DePover and Godfraind, C16-esters might bind to the same receptor site as the C17 side group.[4] Studies are in progress to help resolve these two possibilities.

A new and unexpected finding is that modification of the steroid portion of a given cardenolide glycoside (especially C16β) may modify the effect of altering the C3-OH substituent. It is possible that some interaction via the C16 binding site may induce a conformational effect on the sugar binding site of the receptor. It is also conceivable that if the intrinsic activity of a genin is quite high, then the maximum apparent potentiation caused by addition of a C3-OH sugar would be correspondingly reduced.

Experimental Section

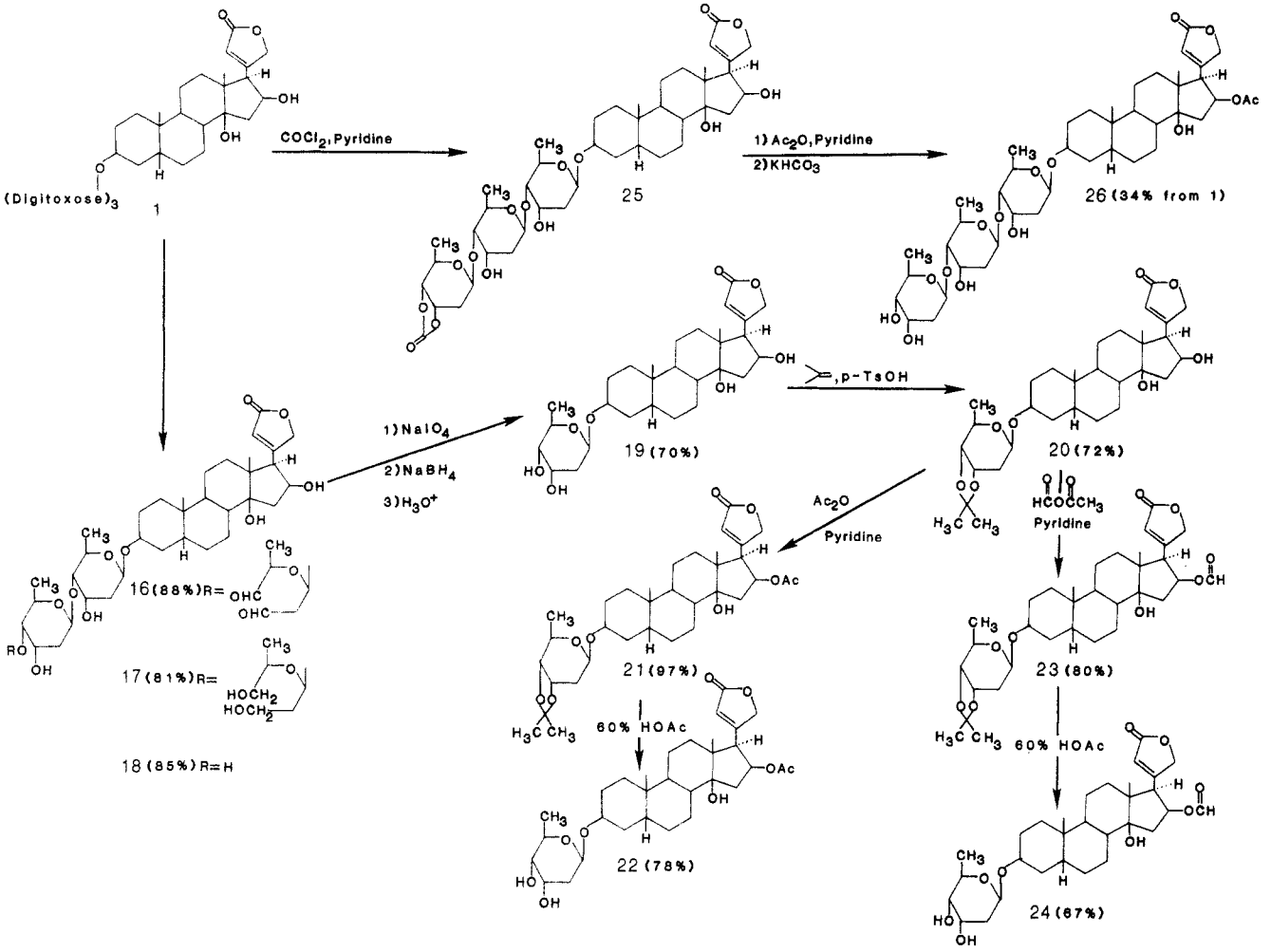
Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. The 80-MHz ^1H NMR and 400-MHz ^1H and ^{13}C spectra were taken at the Oregon State University NMR Spectroscopy Laboratory, Department of Chemistry. The 400-MHz spectra were taken on a Bruker AM 400 spectrometer. IR spectra were run as KBr pellets on a Beckman Model Acculab 7 spectrophotometer. Optical rotations in methanol were taken on a Perkin-Elmer 141 polarimeter. TLC was performed on 0.25-mm EM silica gel 60 F-254 glass plates. Flash column chromatography used silica gel 60, 230–400 mesh (EM Merck), in a 4 × 20 cm column.

(3β,5β,14β,16β,17β)-3,14,16-Trihydroxycard-20(22)-enolide (2) (Gitoxigenin). Gitoxin (**1**), 30 g (0.0384 mol), in 1.5 L of reagent methanol was heated on a steam bath for 15 min and then

Table II. Na^+,K^+-ATPase Inhibition Studies

analogue	hog kidney Na^+,K^+-ATPase inhbn data: K_{50}, M	approx rel act.
digitoxigenin	1.2×10^{-7}	5
gitoxigenin (**2**)	6.03×10^{-7}	1
gitoxigenin 3β-digitoxoside (**19**)	3.80×10^{-8}	15
gitoxigenin 3β-bisdigitoxoside (**18**)	5.19×10^{-8}	12
gitoxin (**1**)	6.99×10^{-8}	10
gitoxigenin 16β-formate (**6**)	2.00×10^{-8}	30
gitoxigenin 16β-formate 3β-digitoxoside (**24**)	3.13×10^{-9}	200
gitoxigenin 16β-formate 3β-acetate (**9**)	1.70×10^{-8}	35
gitoxigenin 3β,16β-diformate (**8**)	1.48×10^{-8}	40
gitoxigenin 3β-formate (**7**)	6.76×10^{-7}	1
gitoxigenin 3β-acetate (**4**)	6.76×10^{-7}	1
gitoxigenin 16β-acetate	4.9×10^{-8}	12
gitoxigenin 3β,16β-diacetate (**3**)	7.41×10^{-8}	8
gitoxigenin 16β-acetate 3β-formate (**10**)	9.55×10^{-8}	6
gitoxigenin 16β-acetate 3β-digitoxoside (**22**)	7.24×10^{-9}	85
gitoxigenin 3β-methoxycarbonate (**11**)	6.03×10^{-7}	1
gitoxigenin 3β,16β-dimethoxycarbonate (**12**)	3.89×10^{-6}	0.2
gitoxin 16β-acetate (**26**)	1.02×10^{-8}	60
gitoxigenin-16-one 3β-acetate (**14**)	8.7×10^{-6}	0.1
17α-gitoxigenin-16-one 3β-acetate (**15**)	7.85×10^{-6}	0.1

Scheme II



1.0 L of 0.08 N H_2SO_4 was added and the solution heated for an additional 2.5 h. The reaction was followed by TLC (CH_2Cl_2–acetone, 2:1). The flask was cooled in ice, 200 mL of 5% $NaHCO_3$ was added, and the resulting suspension was concentrated to 500 mL in vacuo and placed in a freezer at −40 °C overnight. The resulting crstals of **2** were recrystallized in MeOH and $CHCl_3$, yield 10.16 g (68%), mp 223–225 °C (lit.[17] mp 224–226 °C).

(3β,5β,14β,16β,17β)-3,16-Diacetoxy-14-hydroxycard-20-(22)-enolide (3) (Gitoxigenin 3β,16β-Diacetate). Acetic anhydride (50 mL) was added to a stirred solution of gitoxigenin (**2**) (10.05 g) in dry pyridine (60 mL) at room temperature. After stirring for 14 h, the reaction mixture was poured into water and stirred for 5 h at 0–5 °C. The crystals were filtered and recrystallized from MeOH–EtOAc to yield **3** (11.53 g, 90%): mp 247–250 °C (lit.[17] mp 248–250 °C); $[\alpha]^{22}_D$ −28.57° (*c* 1.05, pyridine); IR (KBr) 3550 (OH), 1780, 1745, 1725 (C=O), 1635, 1655 (C=C) cm^{-1}; UV λ_{max} (MeOH) 219 nm (ε 13 744); ^1H NMR (acetone-d_6) δ 5.93 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.45 (1 H, ddd, *J* = 10, 10, 2 Hz, C_{16}-H) 5.05 (1 H, m, C_3-H), 4.81, 5.09 (2 H, dd, *J* = 16, 2 Hz, C_{21}-H), 3.23 (1 H, d, *J* = 10 Hz, C_{17}-H), 2.75 (1 H, dd, *J* = 16, 10 Hz, C_{15}-H), 1.82 (1 H, m, C_{15}-H), 1.94, 2.03 (6 H, s, 2 $OCOCH_3$), 0.98 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{27}H_{38}O_7$) C, H.

(3β,5β,14β,16β,17β)-3-Acetoxy-14,16-dihydroxycard-20-(22)-enolide (4) (Gitoxigenin 3β-Acetate). (a) Method A: Hydrolysis of 3β,16β-Diacetate 3 with $KHCO_3$. To a solution of **3** (9.0 g) in MeOH (2.7 L) was added a solution of $KHCO_3$ (5.4 g) in MeOH (2.16 L)–H_2O (0.54 L), and the mixture was stirred at room temperature for 4 days. HCl (0.05 N) was added dropwise to the stirred solution until pH 6.5 at 0–5 °C. The solution was concentrated in vacuo and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried, and evaporated in vacuo. The residue (8.73 g) was chromatographed on a silica gel column (CH_2Cl_2, with an increasing acetone content). Acetate **4** was eluted with acetone–CH_2Cl_2 (2:8) and recrystallized from MeOH: 5.33 g (65%); mp 228–232 °C (lit.[13] mp 228–233 °C); $[\alpha]^{22}_D$ −3.49° (*c* 0.86, pyridine); IR (KBr) 3560, 3510, 3420, 3350 (OH), 1790, 1765, 1740 (C=O), 1635 (C=C) cm^{-1}; UV λ_{max} (MeOH) 220 nm (ε 14 136); ^1H NMR ($CDCl_3$) δ 5.90 (1 H, s, C_{22}-H), 5.03 (1 H, m, C_3-H), 4.98 (2 H, d, *J* = 16 Hz, C_{21}-H), 3.63 (1 H, d, *J* = 8 Hz, C_{17}-H), 2.85, 2.93 (2 H, s, C_{14}-OH and C_{16}-OH), 2.03 (3 H, s, $OCOCH_3$), 0.98 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{25}H_{36}O_6$) C, H.

(b) Method B: Acetylation of Gitoxigenin (2) with Acetic Anhydride and Acetic Acid. To a solution of **2** (8.00 g) in $CHCl_3$ (100 mL) was added Ac_2O (160 mL) and acetic acid (8 mL). It was stirred at 60–70 °C for 2 h, poured into ice water, and extracted with $CHCl_3$ (300 mL). The $CHCl_3$ was washed successively with H_2O, 5% $NaHCO_3$, and H_2O, dried, and evaporated in vacuo. The residue (8.10 g) was chromatographed (silica gel (200 g, with acetone–CH_2Cl_2 as eluent with acetone content increasing to 15%). After crystallization (MeOH), 1.72 g (17%) of diacetate **3** was obtained, mp 247–250 °C (lit.[13] mp 248–250 °C).

The eluate with 20% acetone–CH_2Cl_2 contained 3β-acetate **4**, which was recrystallized from MeOH, yielding 5.046 g, (56%), mp 229–232 °C (lit.[13] mp 228–235 °C).

(c) Method C: Acetylation of 2 with Acetic Anhydride. A solution of **2** (0.1 g, 2.6 mmol) in Ac_2O (10 mL) was stirred at 60 °C for 2 h. The mixture was concentrated to one-third the original volume in vacuum at 40 °C, H_2O (100 mL) was added, and the mixture was extracted with CH_2Cl_2. The combined extract was washed with 5% aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and concentrated to an amorphous powder. The crude amorphous powder was chromatographed, eluant 10% MeOH–CH_2Cl_2. Acetate **4** (R_f 0.55) was isolated and recrystallized by MeOH to afford 0.05 g (47%) of a white crystalline solid: mp 228–233 °C (lit.[19] mp 228–232 °C). The minor product **3**, R_f 0.75,

(17) Hunger, A.; Reichstein, T. *Helv. Chim. Acta* **1950**, *33*, 76.

was isolated by eluting with 3% MeOH–CH_2Cl_2 and recrystallized by MeOH to afford 0.015 g (10%) of pure **3**; mp 247–259 °C (lit.[19] mp 248–250 °C).

(3β,5β,14β,16β,17β)-16-Acetoxy-3,14-dihydroxycard-20(22)-enolide (5) (Gitoxigenin 16β-Acetate). To a solution of **2** (1.018 g) in dry pyridine (10 mL) was added acetic anhydride (2 mL), and the mixture was stirred at room temperature for 1 h, poured into water, and extracted with $CHCl_3$. The $CHCl_3$ was washed with 1 N HCl, H_2O, 5% $NaHCO_3$, and H_2O, dried, and evaporated in vacuo. The residue (1.05 g) was chromatographed on a silica gel (50 g) column (MeOH–CH_2Cl_2 as eluent with an increasing MeOH content). The product (obtained in the MeOH–CH_2Cl_2 (2.5:7.5 fractions) was recrystallized from MeOH, yielding gitoxigenin 3β,16β-diacetate (**3**) (294 mg, 23%), mp 247–250 °C (lit.[17] mp 248–250 °C). The fractions with MeOH–CH_2Cl_2 (5:9.5) were recrystallized from EtOAc, yielding gitoxigenin 16β-acetate (**5**) (812 mg, 72%): mp 227–229 °C (lit.[13,14] mp 226–229 °C); $[\alpha]^{22}_D$ −26.1° (*c* 1.11, pyridine); IR (KBr) 3525, 3390 (OH), 1795, 1755 (C=O), 1617 (C=C) cm^{-1}; UV λ_{max} (MeOH) 219 nm (ε 12710); ^1H NMR (acetone-d_6) δ 5.90 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.45 (1 H, ddd, *J* = 9, 9, 2 Hz, C_{16}-H), 4.94 (2 H, dd, H = 16, 2 Hz, C_{21}-H), 4.03 (1 H, m, C_3-H), 3.21 (1 H, d, *J* = 9 Hz, C_{17}-H), 2.98 (1 H, s, 14-OH), 2.78 (1 H, dd, *J* = 16, 10 Hz, C_{15a}-H), 1.81 (1 H, dd, *J* = 16, 9 Hz, C_{15b}-H), 1.93 (3 H, s, $OOCCH_3$), 0.98 (3 H, s, C_{19}-H), 0.93 (3 H, s, C_{18}-H). Anal. ($C_{25}H_{36}O_6$) C, H.

(3β,5β,14β,16β,17β)-3,14-Dihydroxy-16-(formyloxy)card-20(22)-enolide (6) (Gitoxigenin 16β-Formate, Gitaloxigenin), (3β,5β,14β,16β,17β)-14,16-Dihydroxy-3-(formyloxy)card-20(22)-enolide (7) (Gitoxigenin 3β-Formate), and (3β,5β,14β,16β,17β)-3,16-Bis(formyloxy)-14-hydroxycard-20(22)-enolide (8) (Gitoxigenin 3β,16β-Diformate). a. Acetic Formic Anhydride. To a dry 1-L, three-necked, round-bottomed flask were added 100 g of sodium formate and 200 mL of anhydrous ether. To this stirred mixture was added 90 mL of distilled acetyl chloride for 5 min, while the temperature was maintained at 23–27 °C. After the addition was complete, the mixture was stirred for 6 h at 23–27 °C and filtered, the solid residue rinsed with 100 mL of dry ether, and the ether removed in vacuo at 0–5 °C. The crude product was distilled to yield 73.5 g (65%) of acetic formic anhydride as a colorless liquid: bp 27–28 °C (10 mmHg) (lit.[18] bp 25–28 °C); IR (neat) 1210, 1750 (C=O), 1060 (C—O—C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.7 (s, 1 H, CH=O), 2.1 (s, 3 H, CH_3).

b. Synthesis of Compounds 6–8. To a solution of 3.017 g of **2** in 70 mL of dry dimethylformamide were added 22.5 mL of acetic formic anhydride and 300 mg of sodium formate. After stirring at room temperature for 1 h, the reaction mixture was poured into ice–H_2O, extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated in vacuo to give an oil (3.10 g). The crude oil was chromatographed (silica gel, 200 g) with acetone–CH_2Cl_2. The eluant of 15% acetone–CH_2Cl_2 was evaporated. Recrystallization (EtOAc) gave 652 mg (18%) of **8** as colorless needles: mp 222–224 °C (lit.[19] mp 221–224 °C); $[\alpha]^{22}_D$ −21.85° (*c* 1.19, pyridine); UV λ_{max} (MeOH) 218 nm (ε 15332); IR (KBr) 3500 (OH), 1795, 1740, 1725, 1700 (C=O), 1632 (C=C) cm^{-1}; ^1H NMR (pyridine-d_5) δ 8.18, 8.35 (2 H, s, CHO), 6.30 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.88 (1 H, m, C_3-H), 5.65 (1 H, m, C_{16}-H), 5.28 (2 H, m, C_{21}-H), 3.43 (1 H, d, *J* = 9 Hz, C_{17}-H), 2.84 (1 H, dd, *J* = 16, 9 Hz, C_{15}-H), 0.89 (3 H, s, C_{18}-H), 1.09 (3 H, s, C_{19}-H). Anal. ($C_{25}H_{34}O_7$) C, H.

The eluant with 20% acetone–CH_2Cl_2 was evaporated. Recrystallization (EtOAc) gave 445 mg (13%) of **7** as colorless needles: mp 207–210 °C (lit.[19] mp 207–210 °C); $[\alpha]^{22}_D$ −5.1° (*c* 0.98, pyridine); UV λ_{max} (MeOH) 220 nm (ε 14888); IR (KBr) 3550, 3500, 3425, 3340 (OH), 1790, 1765, 1730, 1715 (C=O), 1632 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.03 (1 H, s, C_3-OOCH), 5.93 (1 H, s, C_{22}-H), 5.20 (1 H, m, C_3-H), 4.98 (2 H, m, C_{21}-H), 4.50 (1 H, m, C_{16}-H), 3.60 (1 H, d, J = 8 Hz, C_{17}-H), 2.81, 2.93 (2 H, s, C_{14}-OH and C_{16}-OH), 2.43 (1 H, dd, *J* = 16, 7 Hz, C_{15}-H), 0.98 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{24}H_{34}O_6$) C, H.

The eluant of 25% acetone–CH_2Cl_2 was evaporated. Recrystallization gave 1.476 g (45%) of **6** as colorless plates: mp 214–216 °C (lit.[19] mp 215–217 °C); $[\alpha]^{22}_D$ −16.47° (*c* 0.85, pyridine); IR (KBr) 3550, 3430 (OH), 1795, 1740, 1710 (C=O), 1630 (C=C) cm^{-1}; UV λ_{max} (MeOH) 218 nm (ε 14844); ^1H NMR (acetone-d_6) δ 7.93 (1 H, s, OCHO), 5.90 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.58 (1 H, ddd, *J* = 9, 9, 2 Hz, C_{16}-H), 4.80, 5.05 (2 H, dd, *J* = 16, 2 Hz, C_{21}-H_2), 4.05 (1 H, m, C_3-H), 3.28 (1 H, d, *J* = 9 Hz, C_{17}-H), 2.98, 3.23 (2 H, s, C_3-OH and C_{14}-OH), 2.80 (1 H, dd, *J* = 16, 9 Hz, C_{15}-H), 0.95 (3 H, s, C_{18}-H), 1.00 (3 H, s, C_{19}-H). Anal. ($C_{24}H_{34}O_6$) C, H.

c. Alternate Synthesis of 8. To a solution of gitoxigenin (**2**) (1.017 g) in dry pyridine (10 mL) was added acetic formic anhydride (5 mL), and the mixture was stirred at 0–5 °C for 1 h. The reaction mixture was poured into water, stirred for 30 min, and filtered, and the crude crystals were recrystallized (MeOH–EtOAc) to yield 932 mg (80%) of **8** as colorless needles, mp 222–224 °C (lit.[19] mp 221–224 °C).

(3β,5β,14β,16β,17β)-3-Acetoxy-16-(formyloxy)-14-hydroxycard-20(22)-enolide (9) (Gitoxigenin 3β-Acetate 16β-Formate). Method A. To a stirred mixture of **4** (0.3 g, 0.7 mmol), 2,6-bis(methylamino)pyridine (0.1 g), and Et_3N (0.6 mL) in CH_2Cl_2 (5 mL) was slowly added formic acetic anhydride (2 mL, 23 mmol) at 0 °C. The mixture was stirred for 20 min (0–10 °C), added to ice–H_2O, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with 5% aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and concentrated to an oil. The crude oil was chromatographed (eluant 10% EtOAc–CH_2Cl_2) to yield 0.305 g (96%) of pure **9**, recrystalized (MeOH) to white crystalline plates: mp 240–241 °C; $[\alpha]^{22}_D$ −17.86° (*c* 1.40, pyridine); UV λ_{max} (MeOH) 218.5 (log ε 4.16); IR (KBr) 3300 (OH), 2940 (CH), 1730 (α,β-unsaturated C=O), 1760 (formyl, C=O), 1615 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9 (1 H, s, CHO), 5.95 (1 H, s, C_{22}-H), 5.6 (1 H, dd, C_{16}-H), 4.9 (2 H, dd, $J_{21,22}$ = 16 Hz, C_{21}-H), 3.45 (1 H, d, C_{17}-H), 2.84 (1 H, dd, *J* = 16, 9 Hz, C_{15}-H), 2.03 (3 H, s, acetate), 0.99 (6 H, s, C_{18}- and C_{19}-CH_3), ^1H NMR (pyridine-d_5) δ 8.15 (1 H, s, C_{16}-OCH), 6.28 (1 H, s, C_{22}-H), 5.76 (1 H, m, C_3-H), 5.33 (2 H, m, C_{21}-H), 3.41 (1 H, d, C_{17}-H), 2.84 (1 H, dd, *J* = 16, 9 Hz, C_{15}-H), 2.08 (3 H, s, $OCOCH_3$), 0.90 (3 H, s, C_{18}-H), 1.09 (3 H, s, C19-H). Anal. ($C_{26}H_{30}O_7$) C, H.

Method B. To a stirred mixture of **4** (0.15 g, 0.35 mmol), DMAP (0.1 g), and Et_3N (0.8 mL) in dioxane (10 mL) was added formic acid (3 mL) and acetic anhydride (2 mL) at 22 °C. The mixture was then refluxed at 90 °C for 45 min, cooled, added to ice–H_2O, and extracted with CH_2Cl_2. The combined extract was washed with 5% aqueous $NaHCO_3$, 1 N HCl and H_2O, dried over $MgSO_4$, and concentrated to an oil, which was chromatographed (eluant 5% EtOAc–CH_2Cl_2) to yield 0.075 g (49%) of pure **9** along with diacetyl derivative **3** (50%).

Method C. To a solution of 300 mg of 3-acetylgitoxigenin (**4**) in dry pyridine (5 mL) was added acetic formic anhydride (2 mL). It was left at 0–5 °C for 2 h and then poured into ice water and extracted with $CHCl_3$. The chloroform layer was washed successively with 1 N HCl, H_2O, 5% $NaHCO_3$, and H_2O, dried, and evaporated. The residue was recyrstallized (MeOH) to give 275 mg (86%) of **9** as colorless needles, mp 241–242 °C.

(3β,5β,14β,16β,17β)-16-Acetoxy-3-(formyloxy)-14-hydroxycard-20(22)-enolide (10) (Gitoxigenin 3β-Formate 16β-Acetate). To a solution of 580 mg of 16-acetylgitoxigenin (**5**) in pyridine (10 mL) was added acetic formic anhydride (4 mL) at 0–5 °C. The resultant solution was stirred at 0–5 °C for 2 h, added to ice–H_2O, and extracted with $CHCl_3$ (2 × 100 mL). The $CHCl_3$ extract was washed with 1 N HCl, H_2O, 5% $NaHCO_3$, and H_2O, dried over $MgSO_4$, and evaporated to an oil. The crude oil was purified via flash chromatography (CH_2Cl_2, 5% EtOAc–CH_2Cl_2, 10% EtOAc–CH_2Cl_2) to yield 521 mg (84.4%) of **10** as colorless needles: mp 224–227 °C; $[\alpha]^{22}_D$ −39.77° (*c* 0.88, pyridine); IR (KBr) 3525 (OH), 1785, 1745, 1710 (C=O), 1630 (C=C) cm^{-1}; UV λ_{max} (MeOH) 218 nm (ε 14241); ^1H NMR ($CDCl_3$) δ 8.03 (1 H, s, C_3-OCHO), 5.90 (1 H, s, C_{22}-H), 5.45 (1 H, ddd, *J* = 9, 9, 2 Hz, C_{16}-H), 5.20 (1 H, m, C_3-H), 5.03, 4.78 (2 H, d, *J* = 16 Hz, C_{21}-H), 3.18 (1 H, d, *J* = 9 Hz, C_{17}-H), 2.70 (1 H, dd, *J* = 16, 10 Hz, C_{15}-H), 1.95 (3 H, s, $OCOCH_3$), 0.95, 0.93 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{25}H_{34}O_7$) C, H.

(3β,5β,14β,16β,17β)-14,16-Dihydroxy-3-[(methoxycarbonyl)oxy]card-20(22)-enolide (11) (Gitoxigenin 3-Methoxycarbonate) and (3β,5β,14β,16β,17β)-14-Hydroxy-3,16-bis(methoxycarbonyl)oxy]card-20(22)-enolide (12) (Gitoxigenin 3,16-Dimethoxycarbonate). To a solution of 2.00

(18) Krimen, L. I. *Org. Synth.* **1970**, *51*, 1.
(19) Haack, E.; Kaiser, F.; Spingler, H. *Chem. Ber.* **1956**, *89*, 1353.

g of gitoxigenin (2) in pyridine (30 mL) was added $ClCOOCH_3$ (6 mL) at 0–5 °C. It was stirred at 0–5 °C for 6 h, poured into ice–H_2O, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 1 N HCl, H_2O, 5% $NaHCO_3$, and H_2O, dried over $MgSO_4$, and evaporated to an oil (2.05 g). The crude oil was purified via flash chromatography on silica gel (20% EtOAc–CH_2Cl_2). The fractions with **12** (R_f 0.55) were recrystallized (MeOH) to give 0.30 g (11%) of **12**: mp 213–214.5 °C; $[\alpha]^{22}_D$ −15.29° (*c* 0.85, pyridine); IR (KBr) 3550 (OH), 1780, 1745, 1735 (C=O), 1650, 1635 (C=C); UV λ_{max} (MeOH) 218.5 nm (ϵ 14685); ^1H NMR ($CDCl_3$) δ 5.94 (1 H, s, C_{22}-H), 5.35 (1 H, ddd, J = 9, 9, 2 Hz, C_{16}-H), 4.95 (2 H, m, C_{21}-H), 4.95 (1 H, m, C_3-H), 3.76 (3 H, s, C_3-$OCOOCH_3$), 3.69 (3 H, s, C_{16}-$OCOOCH_3$), 3.13 (1 H, d, J = 9 Hz, C_{17}-H), 2.73 (1 H, dd, J = 16, 9 Hz, C_{15}-H), 0.95 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{27}H_{38}O_9$) C, H.

The fractions with R_f 0.25 were recrystallized (MeOH) to give 0.83 g (33.0%) of **11**: mp 213–214.5 °C; $[\alpha]^{22}_D$ +2.78° (*c* 1.08, pyridine); IR (KBr) 3520, 3425 (OH), 1790, 1750, 1730 (C=O), 1620 (C=C) cm^{-1}; UV λ_{max} (MeOH) 220.5 nm (ϵ 15018); ^1H NMR (pyridine-d_6) δ 6.19 (1 H, s, C_{21}-H), 5.58 (2 H, m, C_{22}-H), 5.06 (1 H, m, C_3-H), 4.95 (1 H, m, C_{16}-H), 3.75 (3 H, s, C_3-$OCOOCH_3$), 3.25 (1 H, d, J = 8 Hz, C_{17}-H), 2.70 (1 H, dd, J = 15, 8 Hz, C_{15}-H), 1.11 (3 H, s, C_{19}-H), 0.88 (3 H, s, C_{18}-H). Anal. ($C_{27}H_{38}O_8$) C, H.

In addition, 0.54 g (27%) of gitoxigenin (2) was also recovered.

(3β,5β,14β,16β,17β)-3-Acetoxy-14-hydroxy-16-[(methoxycarbonyl)oxy]card-20(22)-enolide (13) (Gitoxigenin 3β-Acetate 16β-Methoxycarbonate). To a solution of 500 mg of 3-acetylgitoxigenin (4) in pyridine (5 mL) was added $ClCOOCH_3$ (2 mL) at 0–5 °C for 5 h. The reaction mixture was poured into ice–H_2O and extracted with $CHCl_3$. The $CHCl_3$ was washed successively with 1 N HCl and H_2O, dried, and evaporated, and the residue (0.52 g) was dissolved in 30 mL of CH_2Cl_2. To the solution was added 4-(dimethylamino)pyridine (DMAP) (0.1 g), triethylamine (0.6 mL), and $ClCOOCH_3$ (2.0 mL) at 0–5 °C. The solution was stirred at room temperature for 5 h, poured into ice water, and extracted with CH_2Cl_2. The CH_2Cl_2 was washed with H_2O, dried, and evaporated to give an oil. The oil was chromatographed on silica gel (40% EtOAc–CH_2Cl_2) and recrystallized from EtOAc–Et_2O to give 328 mg (57%) of **13**: mp 220–222 °C; $[\alpha]^{22}_D$ −20.20° (*c* 0.99, pyridine); UV λ_{max} (MeOH) 218 nm (ϵ 14446); IR (KBr) 3525 (OH), 1780, 1750, 1720 (C=O), 1635 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.95 (1 H, s, C_{22}-H), 5.35 (1 H, ddd, J = 10, 10, 2 Hz, C_{16}-H), 5.03 (1 H, m, C_3-H), 4.93 (2 H, m, C_{21}-H), 3.68 (3 H, s, C_{16}-$OCOOCH_3$), 3.20 (1 H, d, J = 9 Hz, C_{17}-H), 2.70 (1 H, dd, J = 16, 10 Hz, C_{15}-H), 2.03 (3 H, s, $OCOCH_3$), 0.93, 0.95 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{27}H_{38}O_8$) C, H.

(3β,5β,14β,17β)-3-Acetoxy-14-hydroxy-16-ketocard-20(22)-enolide (14) and (3β,5β,14β,17α)-3-Acetoxy-14-hydroxy-16-ketocard-20(22)-enolide (15). To 1.00 g of 3-acetylgitoxigenin (4) in 70 mL of acetone was added 0.8 mL of Jones reagent (CrO_3–H_2SO_4) at −10 to 0 °C, stirred at −10 to 0 °C for 3 min and then added to ice–H_2O. The quenched reaction was extracted with $CHCl_3$, and the extract was washed with 200 mL of H_2O, dried over $MgSO_4$, and concentrated to crude crystals (1.00 g), which were recrystallized (acetone–Et_2O) to give 525 mg (52%) of **14**: mp 209–211 °C (lit.[20] mp 195–210 °C); $[\alpha]^{22}_D$ +73.0° (*c* 0.97, acetone); IR (KBr) 3550 (OH), 1785, 1748, 1710 (C=O), 1635 (C=C); UV λ_{max} (MeOH) 216 nm (ϵ 10010); ^1H NMR ($CDCl_3$) δ 5.94 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.05 (1 H, m, C_3-H), 4.61, 4.98 (2 H, dd, J = 18 and 2 Hz, C_{21}-H), 3.08 (1 H, s, C_{17}-H), 2.25, 2.89 (2 H, d, J = 18 Hz, C_{15}-H), 2.68 (1 H, s, C_{14}-OH), 2.05 (3 H, s, $OCOCH_3$), 1.00 (3 H, s, C_{18}-H), 1.05 (3 H, s, C_{18}-H). Anal. ($C_{25}H_{34}O_6$) C, H.

The mother liquor was concentrated to dryness (470 mg) and then recrystallized from acetone–Et_2O, giving 107 mg (10.8%) of **15**: mp 232–235 °C (lit.[20] mp 232–235 °C); $[\alpha]^{22}_D$ +131.5° (*c* 0.20, acetone); IR (KBr) 3490 (OH), 1788, 1755 (lactone C=O), 1740 ($OCCH_3$), 1705 (ketone), 1633 (C=C); UV λ_{max} (MeOH) 216 nm (ϵ 9620); ^1H NMR ($CDCl_3$) δ 5.91 (1 H, m, $W_{h/2}$ = 2 Hz, C_{22}-H), 5.03 (1 H, m, C_3-H), 4.64, 4.99 (1 H, dd, J = 18, 2 Hz, C_{21}-H), 3.85 (1 H, s, C_{17}-H), 2.28, 2.68 (2 H, d, J = 18 Hz, C_{15}-H), 2.39 (1 H, s, C_{14}-OH), 2.04 (3 H, s, $OCOCH_3$), 1.25 (3 H, s, C_{18}-H), 1.00 (3 H, s, C_{19}-H). Anal. ($C_{25}H_{34}O_6$) C, H.

(20) Satoh, D.; Morita, J. *Yakugaku Zashi* **1962**, *82*, 156.

(3β,5β,14β,16β,17β)-3-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14,16-dihydroxycard-20(22)-enolide (18) was prepared by using a modification of the Satoh and Aoyama[14] method used for stepwise degradation of digitoxin and gitoxin.

(i) Oxidation of Gitoxin (1) with $NaIO_4$. To a solution of gitoxin (1) (5.0 g, 6.40 mmol) in MeOH (1500 mL) was added a solution of sodium metaperiodate (5 g) in H_2O (50 mL). The resultant mixture was stirred at 22 °C for 2 h and precipitated $NaIO_3$ removed by filtration. The filtrate was concentrated under vacuum to about one-third its original volume and extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with H_2O, dried over $MgSO_4$, and concentrated to give crude dialdehyde **16**, 4.397 g (88%), as a white amorphous powder. It was then recrystallized (acetone/*n*-hexane): mp 183–185 °C; IR (KBr) 3400 (OH), 1725 (α,β-unsaturated C=O), 1740 (CHO), 1620 (C=C) cm^{-1}.

(ii) Reduction of 16 with $NaBH_4$. To a solution of dialdehyde **16** (5.0 g, 6.42 mmol) in MeOH (500 mL) was added $NaBH_4$ (0.59 g) in small portions, and the mixture was stirred for 2 h and then quenched with 5% HOAc to pH 6.5. The mixture was concentrated at 40 °C under vacuum and extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo to give crude dimethylol **17**, 4.503 g (81%), as a white powder: mp 185 °C (acetone/*n*-hexane); IR (KBr) 3450 (OH), 1730 (α,β-unsaturated C=O, 1630 (C=C) cm^{-1}.

(iii) Hydrolysis of 17. To a solution of dimethylol **17** (4.5 g, 5.6 mmol) in MeOH (500 mL) was added with stirring at 22 °C 0.05 N HCl (75 mL) and the reaction mixture stirred for 24 h. The reaction mixture was neutralized with 5% aqueous $NaHCO_3$ to pH 6.5, concentrated in vacuo to one-third its original volume, and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried over $MgSO_4$, and evaporated at 20 °C in vacuo to give crude **18**. It was chromatographed (silica gel, eluant 9% EtOH–CH_2Cl_2) and recrystallized from CH_2Cl_2–*n*-hexane to give pure **18**, yield 3.847 g (85%), as white granular powder: mp 198–201 °C; UV λ_{max} (MeOH) 218 nm; $[\alpha]^{22}_D$ +10.13° (*c* 0.27 MeOH); IR (KBr) 3440 (OH), 1725 (α,β-unsaturated C=O), 1620 (C=C) cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.18 (1 H, s, C_{22}-H), 5.53 (2 H, d, J = 16 Hz, C_{21}-H), 5.37 (1 H, m, C_{16}-H), 4.60 (2 H, m, $C_{3'}$-H and $C_{3''}$-H), 4.35 (1 H, m, $C_{4'}$- and $C_{4''}$-H), 4.19 (1 H, m, C_3-H), 3.48 (2 H, m, $C_{5'}$-H and $C_{5''}$-H), 3.23 (1 H, d, J = 8 Hz, C_{17}-H), 2.68 (2 H, dd, J = 9, 16 Hz, C_{15}-H), 1.39 and 1.44 (6 H, d, J = 6 Hz, $C_{6'}$-H and $C_{6''}$-H), 1.06 (3 H, s, C_{19}-H), 0.83 (3 H, s, C_{18}-H). Anal. ($C_{35}H_{54}O_{11}$) C, H.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14,16-dihydroxycard-20(22)-enolide (19) was prepared from **18** following a similar procedure as used for producing **18** from gitoxin (1). Gitoxigenin bisdigitoxoside (**18**) was oxidized by $NaIO_4$ to the corresponding dialdehyde in 75% yield, which on reduction with $NaBH_4$ and subsequent acid hydrolysis with 0.05 N HCl gave crude gitoxigenin monodigitoxoside **19** in 70% overall yield. The crude **19** was purified by column chromatography, eluant 20% MeOH–CH_2Cl_2 (increasing in MeOH), and recrystallized from EtOH–Et_2O: mp 214–216 °C; $[\alpha]^{22}_D$ +10.13 (*c* 0.27, MeOH); IR (KBr) 3500 (OH), 1730 (α,β-unsaturated C=O), 1615 (C=C) cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.19 (1 H, s, C_{22}-H), 5.54 (2 H, d, J = 16 Hz, C_{21}-H), 4.40 (1 H, m, C_3-H), 4.24 (1 H, m, $C_{3'}$-H), 4.18 (1 H, m, $C_{4'}$-H), 3.57 (1 H, m, $C_{5'}$-H), 3.24 (1 H, d, J = 8 Hz, C_{17}-H), 2.68 (1 H, dd, J = 16, 10 Hz, C_{15}-H), 1.56 (3 H, d, J = 6 Hz, $C_{6'}$-CH_3), 1.08 (3 H, s, C_{19}-H), 0.88 (3 H, s, C_{18}-H). Anal. ($C_{29}H_{44}O_8$) C, H.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-3,4-*O*-(1-methylethylidene)-β-D-*ribo*-hexopyranosyl)oxy]-14,16-dihydroxycard-20(22)-enolide (20). To a solution of **19** (2.5 g, 4.8 mmol) in anhydrous acetone (60 mL) was added *p*-TsOH (25 mg) at 22 °C with stirring. The reaction mixture was stirred for 5 h at 22 °C; by this time the reaction was almost complete as evident from TLC (10% MeOH/CH_2Cl_2). The mixture was concentrated to half the volume in vacuo at 30 °C and to the residue was added H_2O (5 mL). The precipitated solid was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with H_2O and dried over $MgSO_4$. The product was purified by column chromatrography (eluant 10% EtOAc–CH_2Cl_2) to give pure **20**: yield 2.2 g (72%); mp 214–216 °C; $[\alpha]^{22}_D$ +13.12 (*c* 0.15 MeOH); UV λ_{max} (MeOH) 218 nm; IR (KBr) 3490 (OH), 1730 (α,β-unsaturated C=O), 1615 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.91 (1 H, s, C_{22}-H), 4.98 (2 H,

d, J = 16 Hz, C_{21}-H), 4.40 (1 H, m, C_{16}-H), 4.0 (1 H, m, C_3-H), 4.75 (1 H, dd, J = 8, 3 Hz, $C_{1'}$-H), 4.40 (1 H, m, $C_{3'}$-H), 3.53 (1 H, dd, J = 9, 5 Hz, $C_{4'}$-H), 3.48 (1 H, m, $C_{5'}$-H), 2.90 (1 H, m, C_{17}-H), 2.40 (1 H, dd, J = 7, 6 Hz, C_{15}-H), 1.35 and 1.48 (6 H, s, acetonide CH_3), 1.24 (3 H, d, J = 6 Hz, C_6-H), 0.94 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{32}H_{48}O_8$) C, H.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-3,4-*O*-(1-methylethylidene)-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-16-(formyloxy)card-20(22)-enolide (23). Method A. To a stirred mixture of **20** (0.45 g, 0.8 mmol), DMAP (0.2 g), and Et_3N (1.0 mL) in CH_2Cl_2 (10 mL) was slowly added formic acetic anhydride (2.5 mL, 29 mmol) at 10 °C. The mixture was stirred for 30 min, added to ice–H_2O, and extracted with CH_2Cl_2. The combined extract was washed with 5% aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and concentrated to an oil. The crude oil was chromatographed (eluant 10% EtOAc–CH_2Cl_2) to yield 0.3 g (62%) of pure **23**; after recrystallization (EtOAc–Et_2O): mp 197–198 °C; $[\alpha]^{22}_D$ +13.347 (*c* 0.29, MeOH); UV λ_{max} (MeOH) 218 (log ε 4.21); IR (KBr) 3440 (OH), 1765 (C=O), 1730 (α,β-unsaturated C=O), 1620 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9 (1 H, s, CHO), 5.95 (1 H, s, C_{22}-H), 5.60 (1 H, dd, J = 10, 10, 2 Hz, C_{16}-H), 4.94 (2 H, d, J = 16 Hz, C_{21}-H), 4.75 (1 H, dd, J = 8, 3 Hz, $C_{1'}$-H), 4.40 (1 H, m, $C_{3'}$-H), 4.01 (1 H, m, C_3-H), 3.54 (1 H, dd, J = 9, 5 Hz, $C_{4'}$-H), 3.53 (1 H, m, $C_{5'}$-H), 2.78 (1 H, dd, J = 10, 16 Hz, C_{15}-H), 1.36 and 1.49 (6 H, s, acetonide CH_3's), 1.26 (3 H, d, J = 6 Hz, $C_{6'}$-H), 0.98 (3 H, s, C_{18}-H), 0.94 (3 H, s, C_{19}-H). Anal. ($C_{33}H_{48}O_9$) C, H.

Method B. To a solution of **20** (0.50 g) in dry pyridine (6 mL) was added acetic formic anhydride (5 mL) at 0–5 °C, and the mixture was stirred at 0–5 °C for 2 h and poured into ice water, and crystals were filtered. The crystals were recrystallized (EtOAc–Et_2O) to yield 420 mg (80%) of **23** as colorless needles, mp 197–199 °C.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14-hydroxy-16-(formyloxy)card-20(22)-enolide (24). Formate **23** (0.49, 0.68 mmol) was stirred at 22 °C with 60% HOAc (54 mL) for 5 h, monitored with TLC (10% EtOH–CH_2Cl_2). The reaction was poured in ice-cold H_2O and extracted with $CHCl_3$, and the combined $CHCl_3$ extract was washed with H_2O, 5% $NaHCO_3$, and H_2O and dried over $MgSO_4$. The product was concentrated to a crude oil and flash chromatographed (7.5% EtOH-CH_2Cl_2) to yield pure **24** (0.25 g, 67%) as a white amorphous powder: mp 145–150 °C; $[\alpha]^{28}_D$ −14.07° (*c* 0.32, MeOH); UV λ_{max} (MeOH) 218 nm (log ε 4.22); IR (KBr) 3500 (OH), 1740 (CHO), 1710 (α,β-unsaturated C=O), 1615 (C=C); ^1H NMR ($CDCl_3$) δ 7.9 (1 H, s, CHO), 5.95 (1 H, s, C_{22}-H), 5.58 (1 H, ddd, J = 10, 10, 2 Hz, C_{16}-H), 4.95 (2 H, d, J = 16 Hz, C_{21}-H), 4.0 (1 H, m, C_3-H), 4.88 (1 H, dd, J = 8, 2 Hz, $C_{1'}$-H), 4.05 (1 H, m, $C_{3'}$-H), 3.78 (1 H, dd, J = 9, 6 Hz, $C_{4'}$-H), 3.25 (1 H, m, $C_{5'}$-H), 3.23 (1 H, d, J = 9 Hz, C_{17}-H), 1.29 (3 H, d, J = 6 Hz, $C_{6'}$-CH_3). Anal. ($C_{30}H_{44}O_9$) C, H.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-3,4-*O*-(1-methylethylidene)-β-D-*ribo*-hexopyranosyl)oxy]-16-acetoxy-14-hydroxycard-20(22)-enolide (21). To a solution of 0.50 g of **20** in pyridine (6 mL) was added acetic anhydride (6 mL). The resultant solution was stirred at room temperature for 5 h and then added to ice–H_2O. The crystals were filtered and recrystallized (MeOH–Et_2O) to give 449 mg (96%) of **21** as colorless needles: mp 192–195 °C; $[\alpha]^{19}_D$ −7.09 (*c* 0.13, MeOH); IR (KBr) 3400 (OH), 1785, 1760, 1750 (sh) (C=O), 1645, 1625 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.93 (1 H, s, C_{22}-H), 5.45 (1 H, ddd, J = 10, 10, 2 Hz, C_{16}-H), 4.90 (2 H, d, J = 16 Hz, C_{21}-H), 4.73 (1 H, dd, J = 8, 3 Hz, $C_{1'}$-H), 4.38 (1 H, m, $C_{3'}$-H), 3.99 (1 H, m, C_3-H), 3.53 (1 H, dd, J = 9, 5 Hz, $C_{4'}$-H), 3.51 (1 H, m, $C_{5'}$-H), 3.15 (1 H, d, J = 8 Hz, C_{17}-H), 2.70 (1 H, dd, J = 16, 10 Hz, C_{15}-H), 1.95 (1 H, s, C_{16}-OOCCH_3), 1.34, 1.48 (6 H, s, >C(CH_3)$_2$), 1.24 (3 H, d, J = 6 Hz, $C_{6'}$-H), 0.95 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{34}H_{50}O_9$) C, H.

(3β,5β,14β,16β,17β)-3-[(2,6-Dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-16-acetoxy-14-hydroxycard-20(22)-enolide (22). A solution of **21** (200 mg) in 60% AcOH (30 mL) was stirred at room temperature for 5 h, poured into water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried over $MgSO_4$, and evaporated in vacuo to give an oil. The crude oil flash chromatographed (20% acetone–CH_2Cl_2) to yield 146 mg (78%) of **22** as amorphous powder and 41 mg (28.5%) of 16-acetylgitoxigenin (**5**). Data for **22**: mp 167–170 °C; $[\alpha]^{19}_D$ −19.07° (*c* 0.19, MeOH); IR (KBr) 3460 (OH), 1782, 1745 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.95 (1 H, s, C_{22}-H), 5.43 (1 H, ddd, J = 10, 10, 2 Hz, C_{16}-H), 4.90 (2 H, d, J = 16 Hz, C_{21}-H), 4.85 (1 H, dd, J = 8, 2 Hz, $C_{1'}$-H), 4.08 (1 H, m, $C_{3'}$-H), 3.70 (1 H, dd, J = 9, 6 Hz, $C_{4'}$-H), 3.35 (1 H, m, $C_{5'}$-H), 3.18 (1 H, d, J = 9 Hz, C_{17}-H), 1.96 (3 H, s, C_{16}-OCOCH_3), 0.95 (6 H, s, C_{18}-H and C_{19}-H). Anal. ($C_{31}H_{46}O_9$) C, H.

(3β,5β,14β,16β,17β)-3-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-16-acetoxy-14-hydroxycard-20(22)-enolide (Gitoxin 16β-Acetate). To a solution of 2.0 g of gitoxin (**1**) in 100 mL of pyridine was added dropwise 24 mL of a 10% solution of $COCl_2$ in toluene for 30 min at 0–5 °C with shaking for 1 h at about 0 °C. Excess of $COCl_2$ was decomposed by addition of ice water and the reaction product was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 5% HCl, 3% $NaHCO_3$, and H_2O to neutral, dried over anhydrous $MgSO_4$, and evaporated in vacuo to give crude crystals, which were chromatographed (10% MeOH–$CHCl_3$) to yield 1.482 g (71.71%) of gitoxin 3‴,4‴-cyclocarbonate **25** as colorless crystals: mp 238–240 °C dec; $[\alpha]^{22}_D$ +21.6° (*c* 0.52, $CHCl_3$); IR ($CHCl_3$) 3458 (br, OH), 1808 (cyclocarbonyl), 1746 (C=O), 1616 (C=C) cm^{-1}; UV λ_{max} 218 nm (ε 14 590). Anal. ($C_{42}H_{62}O_{15}$) C, H.

To a solution of 1.482 g of **25** in dry pyridine (10 mL) was added acetic anhydride (2 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with $CHCl_3$, and the $CHCl_3$ extract was washed successively with 1 N HCl, H_2O, 5% $NaHCO_3$, H_2O, and dried, and evaporated in vacuo to give 1.656 g of crystals. A solution of the crystals in 150 mL of aqueous acetone (acetone–H_2O, 3:1, v/v) containing 0.4% $KHCO_3$ was allowed to stand at room temperature for 4 days. The resulting solution was neutralized, concentrated in vacuo, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over $MgSO_4$, and evaporated to give 1.454 g of a crude product. The crude product was chromatographed (50% acetone–CH_2Cl_2) and crystallized from acetone to give 527 mg (33.75%) of **26**: mp 238–240 °C; $[\alpha]^{19}_D$ +0.83 (*c* 0.24, MeOH); IR (KBr) 3440 (OH), 1750 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.94 (1 H, s, C_{22}-H), 5.45 (1 H, dd, J = 9, 9 Hz, C_{16}-H), 4.95 (2 H, d, J = 16 Hz, C_{21}-H), 4.23 (1 H, m, 3-H), 1.98 (3 H, s, OCOCH_3), 1.23, 1.30 (9 H, d, J = 6 Hz, $C_{6'}$-H, $C_{6''}$-H, $C_{6'''}$-H), 0.95 (6 H, s, C_{18}-H, C_{19}-H). Anal. ($C_{43}H_{66}O_{15}$) C, H.

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