Studies on Cardiac Ingredients of Plants. XIII[1]: Chemical Modification of Gitoxin to Cardiotonic Compounds without Vascular Contractile Effect

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Nitrated gitoxins (4) and bufotoxin homologues with various lengths of alkyl chain at C-3 of the steroid nucleus (10) were prepared from gitoxin (1). The pharmacological activities of the resulting compounds (4 and 10) were evaluated by measurement of inhibitory effect on Na^+, K^+-adenosine triphosphatase (ATPase) prepared from dog kidney, positive inotropic effect (PIE) on isolated guinea-pig papillary muscle preparations, and the effect on smooth muscle using the mesenteric artery from spontaneously hypertensive rats. Most of the compounds showed a smaller contractile effect on the arterial muscle. Among these compounds, gitoxin 3″-nitrate (4g) exhibited the most desirable biological activities, such as PIE comparable to that of 1, 1.25 times wider concentration-dependent range than 1, and lack of contractile activity on vascular muscle.

Key words gitoxin; nitrate; bufotoxin homologue; Na^+,K^+-ATPase inhibition; positive inotropic effect; arterial relaxing activity

Gitoxin (**1**, Fig. 1) is a cardiac glycoside isolated from *Digitalis purupurea* as one of the major components, together with digitoxin, but it is not clinically used because of its low solubility in water and alcohol and its low uptake from the digestive system. Several chemical modifications of gitoxin (**1**) have been reported, with the aim of obtaining potent cardiac agents.[2)]

We have also performed the chemical modification of cardiac glycosides to obtain potent cardiac agents having no or low side effects, such as vascular contraction. We have so far reported that proscillaridin nitrates show vascular relaxant activity, extension of the concentration-dependent range (CDR) of positive inotropic effect (PIE),[3)] and higher lipophilicity than proscillaridin. Since the guanidyl group of arginine has been reported to be an *in vivo* source of nitric oxide (NO), which acts as the endothelium-derived relaxing factor (EDRF),[4)] we also synthesized bufotoxin homologues of proscillaridin, and found that these compounds cause slight vascular contraction and weak relaxation.[5)] Shimada *et al.* reported various bufotoxin homologues of digitoxin,[6)] but they did not examine the activity of the compounds on smooth muscle. Thus, we attempted to prepare gitoxin nitrates and bufotoxin homologues of gitoxin (**1**). Although gitoxin 16-mononitrate and 3′,3″,3‴,4‴,16-pentanitrate[2c)] have been reported, we were interested in the relation between the biological activity and the positions and numbers of nitrated hydroxyl groups. In this paper, we report the preparation of several mono-, di-, and trinitrates (**4a—g**) and bufotoxin homologues (**10a—j**) from gitoxin (**1**). As cardiac steroids inhibit Na^+,K^+-ATPase, which is considered to induce PIE[7)] by elevation of intracellular

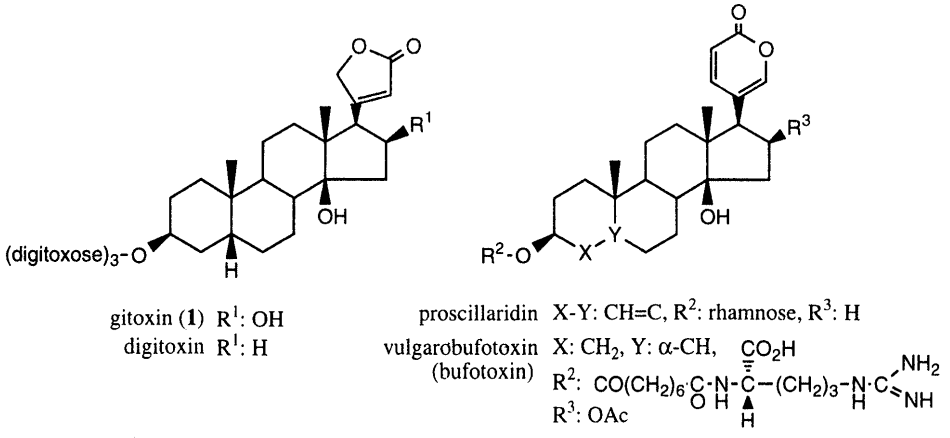

Fig. 1. Structures of Gitoxin (**1**), Digitoxin, Proscillaridin and Vulgarobufotoxin (Bufotoxin)

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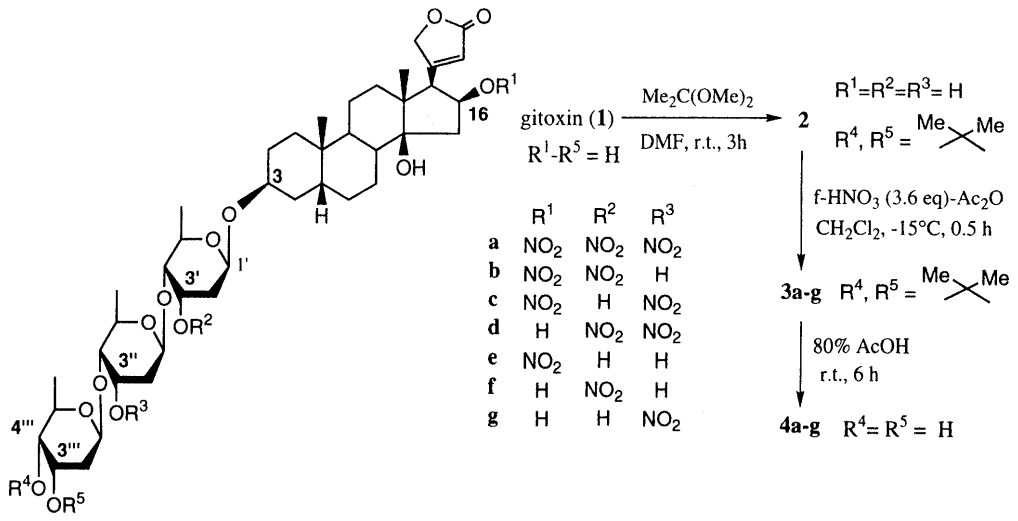

Chart 1

Na^+ concentration, we examined the Na^+,K^+-ATPase-inhibitory activity, and PIE of the compounds. We also report the activity on vascular smooth muscle.

Chemistry Since the direct nitration of gitoxin (**1**) could give 31 nitrates (5 mono-, 10 di-, 10 tri-, 5 tetra-, and 1 pentanitrates), nitration of the protected gitoxin was carried out in order to obtain certain kinds of mono- and dinitrates as shown in Chart 1. The diol at 3‴-C and 4‴-C was protected as an isopropylidene acetal group to give **2**. In the ^{1}H-NMR of **2**, 3‴-H and 4‴-H were appeared at lower field than those of **1**, and two singlet methyl signals due to the isopropylidene acetal group were observed at δ 1.35 and 1.47 ppm. Nitration of **2** was performed in the presence of fuming nitric acid–acetic anhydride mixture to give **3a**—**g** and recovery of **2**. The IR spectrum of **3a** exhibited nitrate absorptions (1640 and 1280 cm^{-1}). In the ^{1}H-NMR of **3a**, 3′-H, 3″-H, and 16-H appeared at lower field (δ 5.67 ppm, 3H, m) than those of **2**, which indicated that **3a** is the 3′,3″,16-trinitrate. The structures of **3d** and **3e** were similarly determined to be the 3′,3″-dinitrate and 16-mononitrate, respectively. Dinitrates (**3b** and **3c**) and mononitrates (**3f** and **3g**) were obtained as mixtures, respectively. As both mixtures were difficult to separate, deprotection was carried out. Compounds **3a**, **3d**, and **3e** and the mixtures (**3b**+**3c** and **3f**+**3g**) were exposed to 80% acetic acid to give the corresponding deprotected nitrates (**4a**, **4d**, **4e**, **4b**+**4c** and **4f**+**4g**) in 80—89% yields. The mixture of **4b**+**4c**, as well as **4f**+**4g**, was separated by reversed-phase HPLC. In the ^{1}H-NMR of **4b** and **4c**, the 3′-H (δ 5.65) and 3″-H (δ 4.22) signals of **4b** appeared at similar positions to those of the H-3″ (δ 5.66) and 3′-H (δ 4.23) signals of **4c**, respectively, as shown in Table 1. In order to determine which is the 3′-nitrate and which is the 3″-nitrate, the nuclear Overhauser effects (NOEs) between 3-H at genins and anomeric 1′-H of **4b** and **4c** were measured, since the signals of the anomeric protons of the 3-*O*-nitrated sugar moiety appeared at *ca.* 0.2 ppm higher field than those of the 3-OH sugar. In the NOE experiments on **4b**, NOE was observed between 3-H and the shifted 1′-H (δ 4.74), while in the case of **4c**, it was observed between 3-H and 1′-H at the original position (δ 4.96) (Fig 2). Thus, **4b** was concluded to be the 3′,16-dinitrate and **4c**, the 3″,16-dinitrate. The structures of **4f** and **4g** were similarly determined as 3′-nitrate and 3″-nitrate, respectively. The nitrates (**4**) showed increased lipophilicity on TLC and improved solubility in alcohol.

Table 1. ^{1}H-NMR Spectral Data for H-3′, H-3″, and H-16 of Gitoxin Nitrates (**4a**—**g**)[a]

4	H-16	H-3′	H-3″
4a		5.66 (3H, m)	
4b	5.65 (2H, m)		4.22 (1H, br s)
4c	5.66[b] (1H, m)	4.23 (1H, br s)	5.66[b] (1H, m)
4d	4.48 (1H, dd, J=7.1, 13.8)	5.64 (2H, m)	
4e	5.66 (1H, ddd, J=2.4, 7.5, 10.8)	4.24 (2H, br s)	
4f	4.48 (1H, dd, J=6.8, 14.5)	5.65 (1H, dd, J=3.2, 6.7)	4.22 (1H, br s)
4g	4.47 (1H, dd, J=6.6, 14.3)	4.23 (1H, br s)	5.67 (1H, dd, J=3.2, 6.5)

a) 400 MHz, $CDCl_3$, δ ppm, (J=Hz). *b*) Overlapping with each other.

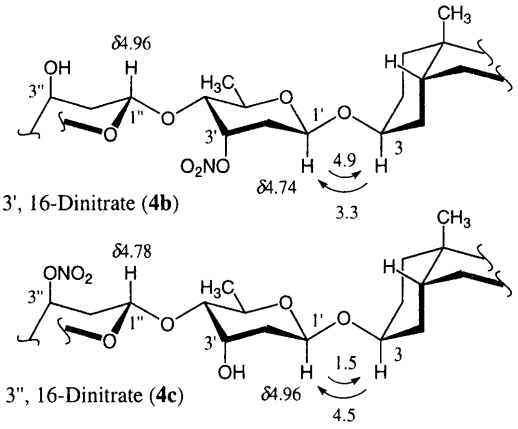

Fig. 2. NOEs (%) Observed in NOE Difference Spectra of **4b** and **4c**

In order to prepare the 3⁗- and 4⁗-mononitrate, we planned to protect the 3′, 3″, and 16-OH groups of **2** as

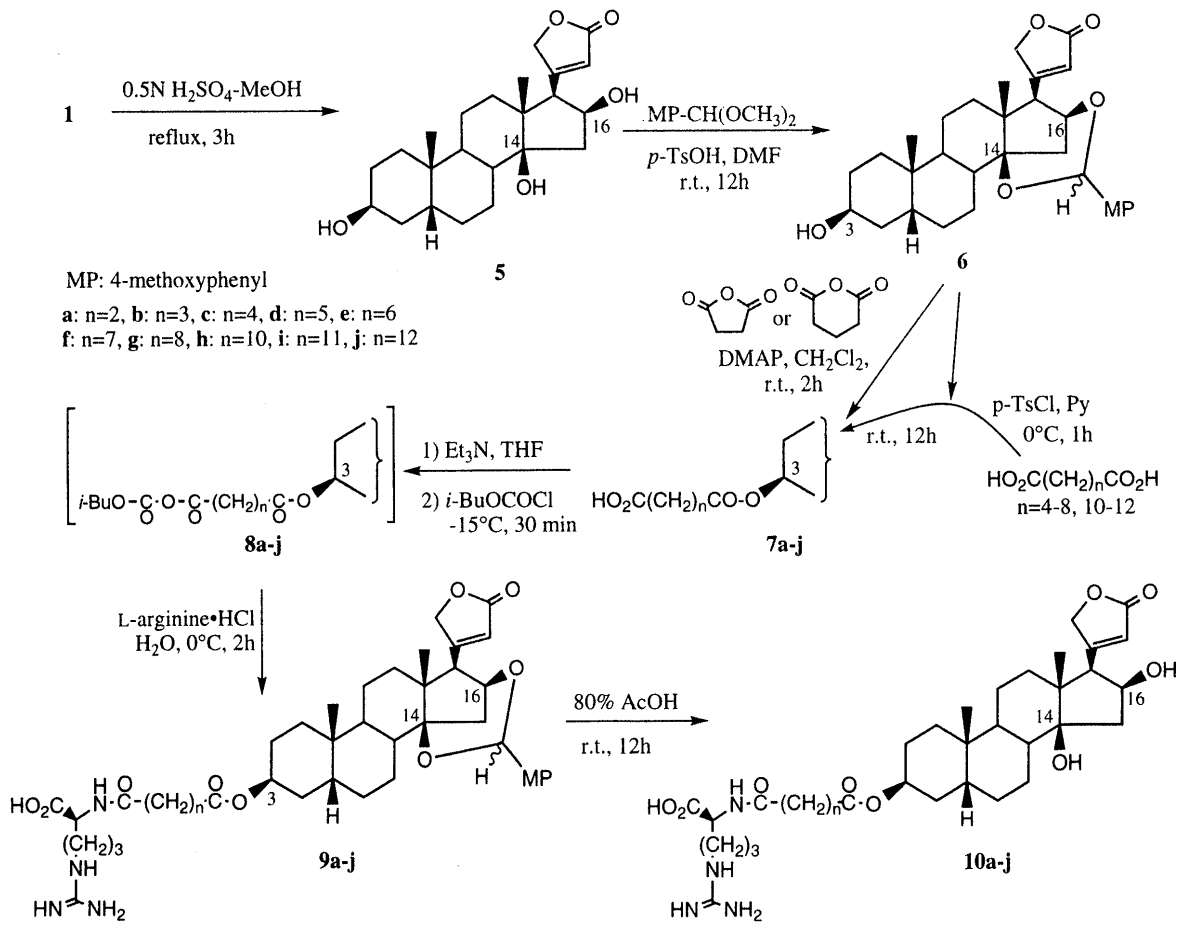

Chart 2

esters, ethers, or silylethers, followed by cleavage of the isopropylidene acetal group, nitration, and deprotection. In every case, however, either protection or deprotection was unsuccessful, because transformations occurred at the sugars or the lactone moieties.

Bufotoxin homologues of gitoxin (**1**) were prepared from protected gitoxingenin (**6**) in almost the same manner as those of proscillaridin (Chart 2).[5] Gitoxin (**1**) was hydrolyzed in acidic condition to gitoxigenin (**5**),[2d] followed by protection of the 1,3-diol at 14-C and 16-C as a *p*-methoxybenzylidene acetal group to give **6** as the mixture of diastereomers. The ^{1}H-NMR spectrum of **6** exhibited the signals of the methoxyl (δ 3.81), phenyl (δ 6.89 and 7.34) and acetal (δ 6.01 for major isomer and δ 6.36 for minor isomer) groups. The signal of 16-H was shifted downfield to δ 4.69. Compound **6** was treated with succinic anhydride to give **7a** in 82% yield. The IR spectrum of **7a** exhibited a broad absorption at 3400 cm^{-1} due to the carboxyl group. In the ^{1}H-NMR spectrum, the signal of 3-H was shifted from δ 4.15 to 5.06. FAB-MS and elemental analysis also supported the structure. Compound **7b** was similarly obtained from **6**. Compounds **7c**—**j** were prepared by the condensation of **6** with the corresponding dicarboxylic acids activated by *p*-toluenesulfonic chloride, as described before.[5] Then, the carboxyl group of **7a** was activated in the presence of isobutyl chloroformate and triethylamine to mixed anhydride (**8a**), followed by addition of L-arginine monohydrochloride to give **9a** in good yield (88%). Compound **9a** gave a positive Sakaguchi test[8] and a negative ninhydrin test, which indicated that **9a** had readily condensed with the α-amino group of L-arginine. The ^{1}H-NMR spectrum of **9a** showed the methine proton signal of the L-arginine residue at δ 4.20 ppm. The structure was also confirmed by FAB-MS and elemental analysis. Compounds **9b**—**j** were similarly obtained in this one-pot reaction from **7b**—**j**. Finally, cleavage of the acetal group gave the desired bufotoxin homologues (**10a**—**j**). Compounds **10a**—**j** also showed improved solubility in alcohol.

Biological Activity and Discussion

The cardiac activity (pIC_{50} and pD_2 values) of **4a**—**g** and **10a**—**j** was examined by assay of the inhibitory activity on Na^+,K^+-ATPase from dog kidney and PIE in isolated guinea-pig papillary muscle.[5,9] The pIC_{50} values indicated that **4a**—**g** are more potent than **1** (Table 2). The pD_2 values of **4e** and **4g** were comparable to that of **1**, and **4c** showed a larger pD_2 value than the other two dinitrates (**4b** and **4d**). These three compounds (**4c**, **4e**, and **4g**) were not nitrated at the 3′-*O* position. These facts suggested that substitution of the 3′-OH group should reduce the PIE, and the 3′-OH group might be important for the binding of the molecule to the enzyme. The development of PIE by compounds **4a** and **4g** occurred over a wider concentration range as compared with **1** (Fig. 3). Compounds **10c**—**j** showed stronger inhibitory activity on Na^+,K^+-ATPase than **1**, and the compounds with longer alkyl chains of the dicarboxylate tended to show

larger pIC_{50} values. This result was very similar to the case of digitoxin derivatives.[6] The pD_2 values of **10f—i** showed the same tendency as the pIC_{50} values, and the pD_2 value of **10i** was comparable to that of **1**. It is noteworthy that **10g—i** exhibited an extremely wide effective concentration range. The efficacy of **10j** was only 1/3 the value of gitoxin (**1**) and a half the value of **10i**. This indicated that too long an alkyl chain disfavors cardiotonic activity, probably because it would become sterically difficult for the molecule to approach Na^+,K^+-ATPase of the heart muscle cells.

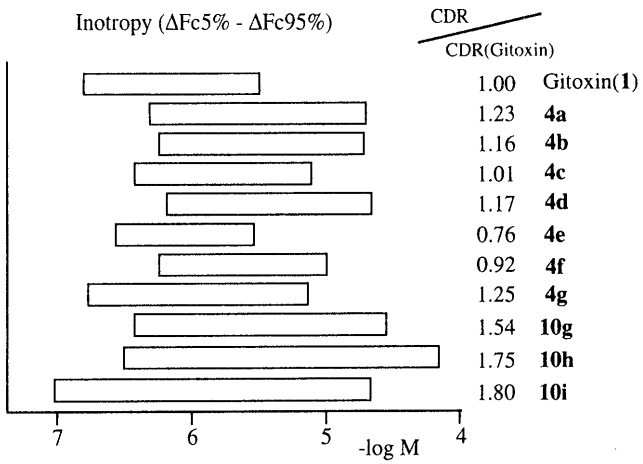

Fig. 3. Concentration-Dependent Range of Gitoxin (**1**), the Nitrates (**4a—g**) and Bufotoxin Homologues (**10g—i**)

The concentration-dependent range was determined by measurement of the range giving from 5 to 95 % of full PIE.

The activity on arterial vascular muscle of **4a—g**, **10a—j**, and **1** was examined using strips of mesenteric artery isolated from 13-week-old spontaneously hypertensive rat (SHR).[5,10] Gitoxin (**1**) at the concentration of 1 and 5 μM and **10f—i** at 1 μM induced mild contraction followed by weak relaxation, while the nitrates (**4c—g**) and 5 μM **10a—j** induced only relaxation (Table 3), probably because the NO radical was generated from the nitro or the guanidyl groups of the derivatives *in vivo* and acted as EDRF.[4] The relaxant activity of **4** and **10** probably cancelled out the contractile effect, so compounds **4** and **10** induced a reduction or disappearance of the contraction. Haustein *et al.* reported that the 16-nitrate (**4e**) induced contraction of the ileum.[2c] These differences were supposed to be due to the tissues used for the measurement. Our results indicate that nitration as well as transformation to bufotoxin homologues is a useful approach to minimize contraction of arteries induced by gitoxin.

In conclusion, we prepared gitoxin nitrates (**4a—g**) and bufotoxin homologues of gitoxin (**10a—j**), and evaluated their cardiotonic activities and the activity on arterial muscle. Most of the compounds showed improved solubility and a wider CDR of the PIE, with only a slight contractile effect on arterial muscle. Among these com-

Table 2. Cardiotonic Activities of Gitoxin (**1**), the Nitrates (**4a—g**), and Bufotoxin Homologues (**10a—j**)

Compound	pIC_{50} ± S.E.[a]	pD_2 ± S.E.[b]	Compound	pIC_{50} + S.E.[a]	pD_2 ± S.E.[b]
Gitoxin (**1**)	6.90±0.01	5.94±0.07	**10a**	>5.00	NT
4a	7.18±0.01	5.47±0.09	**10b**	6.02±0.01	NT
4b	7.48±0.01	5.41±0.03	**10c**	5.92±0.01	NT
4c	7.36±0.01	5.77±0.05	**10d**	6.40±0.01	NT
4d	6.67±0.01	5.38±0.02	**10e**	6.22±0.01	NT
4e	6.87±0.01	5.96±0.06	**10f**	6.66±0.01	4.64, 4.85
4f	6.66±0.01	5.56±0.05	**10g**	6.76±0.01	5.32±0.09
4g	6.66±0.01	5.95±0.07	**10h**	7.28±0.01	5.39±0.09
			10i	6.99±0.01	5.88±0.20
			10j	7.35±0.01	4.6, 7.5

a) pIC_{50} is the concentration of the test compounds required for 50% of the maximum inhibition of the activity of Na^+,K^+-ATPase. The mean±standard error (S.E.) of three experiments. *b*) pD_2 is the concentration of the test compounds required for 50% of the maximum PIE in guinea-pig papillary muscles. The mean±S.E. of three experiments except for **10f** and **10j**. NT, not tested.

Table 3. Arterial Responses Induced by Gitoxin (**1**), the Nitrates (**4a—g**), and Bufotoxin Homologues (**10a—j**)

Compound (*N*)[b]	1 μM (%)[a]		5 μM (%)[a]		Compound (*N*)[b]	1 μM (%)[a]		5 μM (%)[a]	
	Contraction	Relaxation	Contraction	Relaxation		Contraction	Relaxation	Contraction	Relaxation
1 (5)	9.5±2.3	10.6±2.7	4.9±2.2	14.0±3.3	**10a** (5)	ND	1.8±1.1	ND	9.9±3.2
4a (4)	ND	ND	ND	ND	**10b** (5)	ND	1.7±1.3	ND	11.5±3.5
4b (5)	ND	ND	ND	ND	**10c** (5)	ND	2.1±1.5	ND	7.7±2.5
4c (6)	ND	2.2±1.1	ND	18.5±1.2	**10d** (5)	ND	2.5±1.3	ND	6.5±1.8
4d (6)	ND	2.8±1.7	ND	5.2±2.3	**10e** (5)	ND	6.8±2.8	ND	16.1±4.3
4e (6)	ND	2.3±0.8	ND	13.8±2.1	**10f** (5)	5.2±2.3	1.6±1.1	ND	11.0±1.9
4f (6)	ND	2.4±0.7	ND	9.8±2.8	**10g** (5)	13.8±2.1	4.5±1.1	ND	17.3±3.7
4g (6)	ND	2.5±0.7	ND	8.5±2.0	**10h** (4)	9.8±2.8	2.2±1.0	ND	13.6±2.2
DMSO (4)	ND	0.3±0.2	ND	3.0±0.9	**10i** (4)	8.5±2.0	0.5±0.5	ND	10.8±1.3
					10j (4)	ND	3.2±0.8	ND	10.5±2.1

a) Relaxation and contraction induced by the test compounds is expressed as % (means±S.E.) of the relaxation induced by 10^{-4} M papaverine. ND, not detected. *b*) Number of experiments.

pounds, gitoxin 3″-nitrate (**4g**) exhibited the most desirable biological activities, such as PIE comparable to **1**, 1.25 times wider CDR than **1**, and loss of the contractile activity on vascular muscle. Compound **10i** also showed a pD_2 value comparable to that of **1** and an extremely wide CDR of PIE. These findings will be of benefit in the design of more useful gitoxin derivatives.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The FAB-MS were measured with JEOL JMS SX-102 and JEOL JMS DX-505 mass spectrometers, IR spectra with a JASCO IRA-2 spectrometer, and ^{1}H-NMR spectra with a JEOL GSX-400 or JEOL α-500 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used; s, singlet; d, doublet; dd, doublet-of-doublets; ddd, doublet-of-doublet-of-doublets; t, triplet; td, triplet-of-doublet; m, multiplet; br, broad. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. HPLC was carried out using a JASCO 880-PU pump and 830-RI detector. TLC was carried out on precoated plates (Kieselgel $60F_{254}$, 0.25 mm thick, Merck no. 5715), and spots were detected by illumination with an ultraviolet lamp or by spraying 1% $Ce(SO_4)_2$–10% H_2SO_4 followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Davison Chemical Co., Ltd.).

Protection of Gitoxin (1) A suspension of gitoxin (**1**, 500 mg) in *N*,*N*-dimethylformamide (DMF, 25 ml) was stirred with 2,2-dimethoxypropane (3.9 ml) and *p*-toluenesulfonic acid (*p*-TsOH, 30 mg) at room temperature for 3 h. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (MeOH–$CHCl_3$ (1 : 30)) to give **2** (439 mg, 84%).

3β-[(*O*-2,6-Dideoxy-3,4-*O*-isopropylidene-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14β, 16β-dihydroxycard-20(22)-enolide (**2**): Colorless crystalline powder, mp 205—206 °C (MeOH–isopropyl ether), $[\alpha]_D^{25}$ +36.0° (*c* = 0.50, $CHCl_3$). IR (KBr) cm^{-1}: 3500 (OH), 1740 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s, 18-H_3), 0.95 (3H, s, 19-H_3), 1.35, 1.47 (each 3H, both s, $C(CH_3)_2$), 4.02 (1H, br s, 3-H), 4.23 (2H, br s, 3′,3″-H), 4.40 (1H, td, *J* = 2.5, 7.1 Hz, 3‴-H), 4.48 (1H, dd, *J* = 7.1, 14.0 Hz, 16-H), 4.78—4.91 (4H, m, 1′,1″,1‴-H, 21-H), 5.05 (1H, dd, *J* = 1.8, 18.0 Hz, 21-H), 5.96 (1H, t, *J* = 1.8 Hz, 22-H). *Anal.* Calcd for $C_{44}H_{68}O_{14}\cdot H_2O$: C, 62.95; H, 8.41. Found: C, 63.25; H, 8.11.

Nitration of 2 A mixture of fuming HNO_3–Ac_2O (1 : 9, 0.37 ml) was added dropwise to a solution of gitoxin 3‴,4‴-acetonide (**2**, 200 mg) in CH_2Cl_2 (5 ml) at −15 °C and the reaction mixture was stirred at −15 °C for 30 min, then poured into ice-water, and the whole was extracted with AcOEt. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on silica gel (acetone–CH_2Cl_2 (1 : 8—1 : 4)) to give **3a** (32 mg, 14%), **3b** + **3c** (49 mg, 22%), **3d** (29 mg, 13%), **3e** (28 mg, 13%), **3f** + **3g** (46 mg, 22%), and recovery of **2** (27 mg, 14%).

3β-[(*O*-2,6-Dideoxy-3,4-*O*-isopropylidene-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**3a**): mp 155—157 °C (MeOH–isopropyl ether), $[\alpha]_D^{25}$ +28.5° (*c* = 0.50, MeOH). IR (KBr) cm^{-1}: 3500 (OH), 1750 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 1.34, 1.47 (each 3H, both s, $C(CH_3)_2$), 4.02 (1H, br s, 3-H), 4.36 (1H, m, 3‴-H), 4.76 (3H, m, 1′,1″,1‴-H), 4.86 (1H, dd, *J* = 1.8, 18.5 Hz, 21-H), 4.96 (1H, dd, *J* = 1.8, 18.5 Hz, 21-H), 5.67 (3H, m, 3′, 3″, 16-H), 6.06 (1H, t, *J* = 1.8 Hz, 22-H). *Anal.* Calcd for $C_{44}H_{65}N_3O_{20}$: C, 55.26; H, 6.86; N, 4.4. Found: C, 55.38; H, 6.80; N, 4.43.

3b + **3c**: ^{1}H-NMR ($CDCl_3$) δ: 0.93, 0.93 (both s, 18-H_3 of **3b** and **3c**), 0.94 (3H, s, 19-H_3), 1.34, 1.46 (each 3H, both s, $C(CH_3)_2$), 4.02 (1H, br s, 3-H), 4.20, 4.23 (each 1H, both s, 3′-H or 3″-H), 4.37 (1H, m, 3‴-H), 4.73—4.99 (5H, m, 1′,1″,1‴-H, 21-H), 5.66 (2H, m, 3′ or 3″-H, 16-H), 6.06 (1H, t, *J* = 1.8 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-3,4-*O*-isopropylidene-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β,16β-dihydroxycard-20(22)-enolide (**3d**): mp 190—192 °C (MeOH–isopropyl ether), $[\alpha]_D^{25}$ +44.3° (*c* = 0.40, MeOH). IR (KBr) cm^{-1}: 3500 (OH), 1750 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 1.34, 1.46 (each 3H, both s, $C(CH_3)_2$), 4.02 (1H, br s, 3-H), 4.36 (1H, m, 3‴-H), 4.48 (1H, dd, *J* = 6.8, 14.8 Hz, 16-H), 4.77 (3H, m, 1′,1″,1‴-H), 4.88 (1H, dd, *J* = 1.7, 18.0 Hz, 21-H), 5.05 (1H, dd, *J* = 1.7, 18.0 Hz, 21-H), 5.63 (2H, m, 3′,3″-H), 5.97 (1H, t, *J* = 1.7 Hz, 22-H). *Anal.* Calcd for $C_{44}H_{66}N_2O_{18}$: C, 57.99; H, 7.31; N, 3.08. Found: C, 58.13; H, 7.46; N, 2.93.

3β-[(*O*-2,6-Dideoxy-3,4-*O*-isopropylidene-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**3e**): mp 143—145 °C (MeOH–isopropyl ether), $[\alpha]_D^{25}$ +1.4° (*c* = 0.40, MeOH). IR (KBr) cm^{-1}: 3500 (OH), 1750 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 1.34, 1.47 (each 3H, both s, $C(CH_3)_2$), 4.03 (1H, br s, 3-H), 4.23 (2H, br s, 3′,3″-H), 4.40 (1H, m, 3‴-H), 4.79—5.08 (5H, m, 1′,1″,1‴-H, 21-H), 5.66 (1H, ddd, *J* = 2.4, 10.1, 10.6 Hz, 16-H), 6.06 (1H, t, *J* = 1.7 Hz, 22-H). *Anal.* Calcd for $C_{44}H_{67}NO_{16}$: C, 61.01; H, 7.80; N, 1.62. Found: C, 60.89; H, 7.65; N, 1.53.

3f + **3g**: ^{1}H-NMR ($CDCl_3$) δ: 0.91, 0.92 (both s, 18-H_3 of **3f** and **3g**), 0.94 (3H, s, 19-H_3), 1.34, 1.46 (each 3H, both s, $C(CH_3)_2$), 4.02 (1H, br s, 3-H), 4.20, 4.23 (each 1H, both s, 3′-H or 3″-H), 4.37 (1H, m, 3‴-H), 4.48 (1H, m, 16-H), 4.73—5.08 (5H, m, 1′,1″,1‴-H, 21-H), 5.65 (1H, m, 3′ or 3″-H), 5.96 (1H, t, *J* = 1.7 Hz, 22-H).

Cleavage of Isopropylidene Acetal Groups of 3a—g A solution of **3a** (115 mg) in 80% acetic acid (20 ml) was stirred at room temperature. After 6 h, the solvent was removed *in vacuo*. The resulting residue was chromatographed on silica gel (acetone–CH_2Cl_2 (1 : 5)) to give **4a** (97 mg, 89%, 12% from **1**) as a colorless crystalline powder. Compounds **3b—g** were similarly treated to give **4b** + **4c** (82%, 18% from **1**), **4d** (85%, 11% from **1**), **4e** (80%, 10% from **1**), and **4f** + **4g** (86%, 20% from **1**). The separation of **4b** and **4c** was carried out by HPLC (YMC A-324 S-5, 10 mm i.d. × 300 mm, CH_3CN–H_2O (63 : 37), 2 ml/min) to give **4b** (9% from **1**) and **4c** (9% from **1**) as single compounds. The mixture of **4f** (10% from **1**) and **4g** (10% from **1**) were similarly separated using CH_3CN–H_2O (55 : 45) as the HPLC solvent. The physicochemical data of **4a—g** are summarized in Table 4.

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**4a**): IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.02 (1H, br s, 3-H), 4.09 (1H, br s, 3‴-H), 4.76 (2H, m, 1′,1″-H), 4.84—4.99 (3H, m, 1‴-H, 21-H), 5.66 (3H, m, 3′, 3″, 16-H), 6.06 (1H, t, *J* = 1.8 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**4b**): IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.02 (1H, br s, 3-H), 4.12 (1H, br s, 3‴-H), 4.22 (1H, br s, 3″-H), 4.74 (1H, dd, *J* = 1.9, 9.6 Hz, 1′-H), 4.84—4.99 (4H, m, 1″,1‴-H, 21-H), 5.65 (2H, m, 3′, 16-H), 6.07 (1H, t, *J* = 1.8 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**4c**): IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.03 (1H, br s, 3-H), 4.10 (1H, br s, 3‴-H), 4.23 (1H, br s, 3′-H), 4.78 (1H, dd, *J* = 1.9, 9.8 Hz, 1″-H), 4.84—4.99 (4H, m, 1′,1‴-H, 21-H), 5.66 (2H, m, 3″, 16-H), 6.08 (1H, t, *J* = 1.8 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β,16β-dihydroxycard-20(22)-enolide (**4d**): IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.02 (1H, br s, 3-H), 4.08 (1H, br s, 3‴-H), 4.48 (1H, dd, *J* = 7.1, 13.8 Hz, 16-H), 4.75 (2H, m, 1′,1″-H), 4.86—5.08 (3H, m, 1‴-H, 21-H), 5.64 (2H, m, 3′,3″-H), 5.96 (1H, t, *J* = 1.6 Hz, 22-H).

3β-[(*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]-14β-hydroxy-16β-nitroxycard-20(22)-enolide (**4e**)[11]: IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1630, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.03 (1H, br s, 3-H), 4.12

Table 4. Physicochemical Data of Gitoxin Nitrates (**4a—g**) and Bufotoxin Homologues (**10a—j**)

Compound	$[\alpha]_D^{25}$ (*c*) (in MeOH)	mp (°C)	Formula	Analysis (%) Calcd			Found		
				C	H	N	C	H	N
4a	+22.4 (0.50)	161—163[a]	$C_{41}H_{61}N_3O_{20}$	53.75	6.72	4.59	53.67	6.80	4.34
4b	+8.2 (0.50)	158—160[a]	$C_{41}H_{62}N_2O_{18}$	56.53	7.18	3.22	56.33	7.16	3.00
4c	+8.9 (0.35)	154—157[a]	$C_{41}H_{62}N_2O_{18}$	56.53	7.18	3.22	56.35	7.18	3.02
4d	+45.0 (0.30)	199—201[a]	$C_{41}H_{62}N_2O_{18}\cdot 1/2H_2O$	55.95	7.22	3.18	56.19	7.09	3.29
4e	−7.2 (0.50)	163—165[a]	$C_{41}H_{63}NO_{16}\cdot H_2O$	58.33	7.77	1.66	58.58	7.50	1.70
4f	+34.1 (0.50)	221—223[a]	$C_{41}H_{63}NO_{16}$	59.61	7.69	1.70	59.38	7.71	1.75
4g	+34.8 (0.50)	188—190[a]	$C_{41}H_{63}NO_{16}$	59.61	7.69	1.70	59.40	7.66	1.55
10a	+21.2 (0.34)	>260[b]	$C_{33}H_{50}N_4O_9\cdot H_2O$	59.62	7.88	8.43	59.63	7.69	8.70
10b	+55.5 (0.38)	190—192[b]	$C_{34}H_{52}N_4O_9\cdot H_2O$	60.16	8.02	8.25	60.45	7.93	8.25
10c	+16.4 (0.20)	185—187[b]	$C_{35}H_{54}N_4O_9\cdot H_2O$	60.67	8.15	8.09	60.82	8.13	7.91
10d	+21.1 (0.73)	190—192[b]	$C_{36}H_{56}N_4O_9\cdot H_2O$	61.17	8.27	7.93	60.92	8.41	7.76
10e	+19.3 (0.27)	180—182[b]	$C_{37}H_{58}N_4O_9\cdot H_2O$	61.65	8.39	7.77	61.45	8.13	7.52
10f	+14.6 (0.30)	170—172[b]	$C_{38}H_{60}N_4O_9\cdot H_2O$	62.10	8.50	7.62	62.04	8.53	7.91
10g	+15.8 (0.31)	172—174[b]	$C_{39}H_{62}N_4O_9\cdot H_2O$	62.54	8.61	7.48	62.56	8.40	7.29
10h	+16.7 (0.22)	170—172[b]	$C_{41}H_{66}N_4O_9\cdot H_2O$	63.38	8.82	7.21	63.10	8.56	6.94
10i	+18.1 (0.70)	165—167[b]	$C_{42}H_{68}N_4O_9\cdot H_2O$	63.77	8.92	7.08	63.65	8.69	7.25
10j	+14.2 (0.48)	140—142[b]	$C_{43}H_{70}N_4O_9\cdot H_2O$	64.15	9.01	6.96	64.00	9.01	7.26

a) Recrystallizing solvent, MeOH–H_2O. Colorless crystalline powder. *b*) Recrystallizing solvent, MeOH. Colorless crystalline powder.

(1H, br s, 3‴-H), 4.24 (2H, br s, 3′,3″-H), 4.84—5.09 (5H, m, 1′,1″,1‴-H, 21-H), 5.66 (1H, ddd, J=2.4, 7.5, 10.8 Hz, 16-H), 6.06 (1H, t, J=1.8 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl)oxy]-14β,16β-dihydroxycard-20(22)-enolide (**4f**): IR (KBr) cm^{-1}: 3450 (OH), 1735 (C=O), 1630, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.02 (1H, br s, 3-H), 4.12 (1H, br s, 3‴-H), 4.22 (1H, br s, 3″-H), 4.48 (1H, dd, J=6.8, 14.5 Hz, 16-H), 4.74 (1H, dd, J=1.9, 9.4 Hz, 1′-H), 4.85—5.08 (4H, m, 1″,1‴-H, 21-H), 5.65 (1H, dd, J=3.2, 6.7 Hz, 3′-H), 5.97 (1H, t, J=1.7 Hz, 22-H).

3β-[(*O*-2,6-Dideoxy-β-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-3-*O*-nitro-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-14β,16β-dihydroxycard-20(22)-enolide (**4g**): IR (KBr) cm^{-1}: 3500 (OH), 1740 (C=O), 1640, 1280 (ONO_2). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s, 18-H_3), 0.94 (3H, s, 19-H_3), 4.02 (1H, br s, 3-H), 4.10 (1H, br s, 3‴-H), 4.23 (1H, br s, 3′-H), 4.47 (1H, dd, J=6.6, 14.3 Hz, 16-H), 4.78 (1H, dd, J=1.9, 9.7 Hz, 1″-H), 4.84—5.08 (4H, m, 1′,1‴-H, 21-H), 5.67 (1H, dd, J=3.2, 6.5 Hz, 3″-H), 5.97 (1H, t, J=1.7 Hz, 22-H).

Protected Gitoxigenin (6) *p*-Anisaldehyde dimethylacetal (2 ml) and *p*-TsOH (20 mg) were added to a solution of gitoxigenin (**5**, 300 mg) in DMF (20 ml). The mixture was stirred at room temperature for 12 h, poured into cold water, and extracted with AcOEt. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on SiO_2 (CH_2Cl_2–acetone) to give the diastereo mixture of **6** (321 mg, 82%) as a colorless crystalline powder.

3β-Hydroxy-14β,16β-*O*-(4-methoxybenzylidene)card-20(22)-enolide (**6**): IR (KBr) cm^{-1}: 3450 (OH),1750 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.90 (1H, d, J=13.9 Hz, 17-H), 3.81 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.15 (1H, br, 3-H), 4.68—4.73 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.5 Hz , 21-H, major), 4.80—4.97 (3H, m, 16-H, 21-H_2, minor), 5.66 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.01 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.89 (2H, d, J=8.8 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.8 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS *m/z*: 509 $(M+H)^+$. *Anal.* Calcd for $C_{31}H_{40}O_6$: C, 73.20; H, 7.93. Found: C, 73.05; H, 7.93.

Synthesis of 7a,b 4-Dimethylaminopyridine (DMAP, 120 mg, 5 eq) and succinic anhydride (100 mg, 5 eq) were added to a solution of **6** (100 mg, 0.197 mmol) in CH_2Cl_2. The mixture was stirred for 3 h at room temperature, poured into cold water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was chromatographed on SiO_2 (CH_2Cl_2–acetone) to give **7a** (98 mg, 82%) as a colorless crystalline powder. Compound **7b** was similarly obtained from **6** using glutaric anhydride in 96% yield.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Succinate (**7a**): IR (KBr) cm^{-1}: 3450 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.87 (1H, d, J=9.9 Hz, 17-H), 3.80 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.67—4.73 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.82—4.97 (3H, m, 16-H, 21-H_2, minor), 5.06 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.85 (1H, s, 22-H, major), 6.01 (1H, s, CH-Ph-OCH_3, major), 6.34 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS *m/z*: 609 $(M+H)^+$. *Anal.* Calcd for $C_{35}H_{44}O_9$: C, 69.06; H, 7.29. Found: C, 68.91; H, 7.02.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Glutarate (**7b**): IR (KBr) cm^{-1}: 3450 (br, OH, COOH), 1750—1730 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.88 (1H, d, J=12.9 Hz, 17-H), 3.80 (3H, s, OCH_3, major), 3.85 (3H, s, OCH_3, minor), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.82—4.97 (3H, m, 16-H, 21-H_2, minor), 5.06 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.85 (1H, s, 22-H, major), 6.01 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.34 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.88 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS *m/z*: 623 $(M+H)^+$. *Anal.* Calcd for $C_{36}H_{46}O_9$: C, 69.43; H, 7.45. Found: C, 69.07; H, 7.18.

Synthesis of 7c—j A solution of adipic acid (86 mg, 0.59 mmol) in pyridine (10 ml) was treated with *p*-TsCl (225 mg, 1.18 mmol) and the mixture was stirred at 0 °C for 30 min, followed by dropwise addition of a pyridine solution (6 ml) of **6** (100 mg, 0.197 mmol). The reaction mixture was stirred at room temperature for 4 h, poured into water and extracted with EtOAc three times. The organic phase was washed with 5% HCl and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by SiO_2 column chromatography (CH_2Cl_2–acetone) to give **7c** (96 mg, 77%).

Compounds **7d—h** were also prepared from **6** and the corresponding dicarboxylic acids in the same manner.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Adipate (**7c**): IR (KBr) cm^{-1}: 3480 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.90 (1H, d, J=13.6 Hz, 17-H), 3.81 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.80—4.97 (3H, m, 16-H, 21-H_2, minor), 5.12 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.37 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.89 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor) . FAB-MS *m/z*: 637 $(M+H)^+$. *Anal.* Calcd for $C_{37}H_{48}O_9$: C, 69.79; H, 7.60. Found: C, 69.57; H, 7.44.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-

yl Pimelate (**7d**): colorless crystalline powder (85%). IR (KBr) cm^{-1}: 3440 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.91 (1H, d, J=13.5 Hz, 17-H), 3.82 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.70—4.74 (2H, m, 16-H, 21-H, major), 4.87 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.80—4.97 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 651 $(M+H)^+$. *Anal.* Calcd for $C_{38}H_{50}O_9$: C, 70.13; H, 7.44. Found: C, 69.95; H, 7.84.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Suberate (**7e**): colorless crystalline powder (78%). IR (KBr) cm^{-1}: 3450 (br, OH, COOH), 1720—1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.90 (1H, d, J=13.6 Hz, 17-H), 3.81 (3H, s, OCH_3, major), 3.86 (3H, s, OCH_3, minor), 4.70—4.74 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.81—4.97 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.37 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.89 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 665 $(M+H)^+$. *Anal.* Calcd for $C_{39}H_{52}O_9$: C, 70.46; H, 7.88. Found: C, 70.22; H, 7.85.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Azelate (**7f**): Colorless crystalline powder (75%). IR (KBr) cm^{-1}: 3480 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.91 (1H, d, J=13.9 Hz, 17-H), 3.82 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.70—4.76 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=2.0, 18.5 Hz, 21-H, major), 4.82—4.95 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.7 Hz, Ph-2′-H, 6′-H, major+minor), 7.35 (2H, d, J=8.7 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 679 $(M+H)^+$. *Anal.* Calcd for $C_{40}H_{54}O_9$: C, 70.77; H, 8.02. Found: C, 70.71; H, 7.96.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl Sebacoate (**7g**): Colorless crystalline powder (80%). IR (KBr) cm^{-1}: 3450 (br, OH, COOH), 1700—1680 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.91 (1H, d, J=13.9 Hz, 17-H), 3.82 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.70—4.76 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=2.0, 18.8 Hz, 21-H, major), 4.83—4.95 (3H, m, 16-H, 21-H2, minor), 5.10 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.03 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.35 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 693 $(M+H)^+$. *Anal.* Calcd for $C_{41}H_{56}O_9$: C, 71.07; H, 8.15. Found: C, 70.93; H, 8.01.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl 1,10-Decanedicarboxylate (**7h**): Colorless crystalline powder (86%). IR (KBr) cm^{-1}: 3450 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.92 (1H, d, J=13.4 Hz, 17-H), 3.82 (3H, s, OCH_3, major), 3.88 (3H, s, OCH_3, minor), 4.71—4.76 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=1.8, 18.9 Hz, 21-H, major), 4.81—5.01 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.64 (1H, s, 22-H, minor), 5.88 (1H, s, 22-H, major), 6.03 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.7 Hz, Ph-2′-H, 6′-H, major+minor), 7.35 (2H, d, J=8.7 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 721 $(M+H)^+$. *Anal.* Calcd for $C_{43}H_{60}O_9$: C, 71.64; H, 8.39. Found: C, 71.39; H, 8.54.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl 1,11-Undecanedicarboxylate (**7i**): Colorless crystalline powder (88%). IR (KBr) cm^{-1}: 3400 (br, OH, COOH), 1710—1690 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.90 (1H, d, J=13.6 Hz, 17-H), 3.81 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.69—4.74 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.7 Hz, 21-H, major), 4.80—4.95 (3H, m, 16-H, 21-H_2, minor), 5.11 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.89 (2H, d, J=8.8 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.8 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 735 $(M+H)^+$. *Anal.* Calcd for $C_{44}H_{62}O_9$: C, 71.91; H, 8.50. Found: C, 71.80; H, 8.67.

Hydrogen 14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl 1,12-Dodecanedicarboxylate (**7j**): Colorless crystalline powder (81%). IR (KBr) cm^{-1}: 3480 (br, OH, COOH), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.92 (1H, d, J=13.5 Hz, 17-H), 3.82 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.69—4.74 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=1.7, 18.8 Hz, 21-H, major), 4.80—4.95 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.88 (1H, s, 22-H, major), 6.03 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.35 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 749 $(M+H)^+$. *Anal.* Calcd for $C_{45}H_{64}O_9$: C, 72.16; H, 8.61. Found: C, 72.45; H, 8.49.

Synthesis of 9a—j Compound **7a** (53.4 mg, 0.0878 mmol) in tetrahydrofuran (18 ml) was stirred with triethylamine (0.30 ml, 2.11 mmol) at −15 °C for 15 min, followed by dropwise addition of isobutyl chloroformate (57 μl, 5 eq). After 30 min, L-arginine monohydrochloride (77 mg, 5 eq) in water (2 ml) was added dropwise and the reaction mixture was stirred at 0 °C for 2 h. The solvent was removed under reduced pressure, MeOH was added to the residue and the solution was filtered to remove the excess L-arginine. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on silica gel ($CHCl_3$–MeOH–H_2O (7 : 3 : 1), lower phase) to give **9a** (56.8 mg, 85%). Compounds **9b—h** were prepared in the same manner from the corresponding hemiesters (**7b—h**) and L-arginine monohydrochloride.

3-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylpropanoyl-L-arginine (**9a**): IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.53—2.67 (5H, m, 3-$OCOCH_2$, C<u>H_2</u>CONH, 15α-H), 2.90 (1H, d, J=13.4 Hz, 17-H), 3.15 (2H, m, C<u>H_2</u>NHC(NH)NH_2), 3.82 (3H, s, OCH_3, major), 3.84 (3H, s, OCH_3, minor), 4.20 (1H, m, C<u>H</u>(COOH)NH), 4.70—4.75 (2H, m, 16-H, 21-H, major), 4.87 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.85—4.95 (3H, m, 16-H, 21-H_2, minor), 5.09 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.35 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 765 $(M+H)^+$. *Anal.* Calcd for $C_{41}H_{56}N_4O_{10}\cdot H_2O$: C, 62.90; H, 7.47; N, 7.16. Found: C, 62.95; H, 7.39; N, 7.16.

4-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylbutanoyl-L-arginine (**9b**): Colorless crystalline powder (84%). IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1750—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.40—2.62 (5H, m, 3-$OCOCH_2$, C<u>H_2</u>CONH, 15α-H), 2.90 (1H, d, J=13.7 Hz, 17-H), 3.18 (2H, m, C<u>H_2</u>NHC(NH)NH_2), 3.81 (3H, s, OCH_3, major), 3.86 (3H, s, OCH_3, minor), 4.20 (1H, m, C<u>H</u>(COOH)NH), 4.69—4.74 (2H, m, 16-H, 21-H, major), 4.85 (1H, dd, J=1.8, 18.3 Hz, 21-H, major), 4.82—4.97 (3H, m, 16-H, 21-H_2, minor), 5.09 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.35 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 779 $(M+H)^+$. *Anal.* Calcd for $C_{42}H_{58}N_4O_{10}\cdot H_2O$: C, 63.30; H, 7.59; N, 7.03. Found: C, 63.46; H, 7.79; N, 7.29.

5-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylpentanoyl-L-arginine (**9c**): Colorless crystalline powder (84%). IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1750—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.40—2.62 (5H, m, 3-$OCOCH_2$, C<u>H_2</u>CONH, 15α-H), 2.90 (1H, d, J=13.6 Hz, 17-H), 3.18 (2H, m, C<u>H_2</u>NHC(NH)NH_2), 3.81 (3H, s, OCH_3, major), 3.86 (3H, s, OCH_3, minor), 4.16 (1H, m, C<u>H</u>(COOH)NH), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.87 (1H, dd, J=1.8, 18.7 Hz, 21-H, major), 4.80—4.96 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.36 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.90 (2H, d, J=8.9 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.9 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 793 $(M+H)^+$. *Anal.* Calcd for $C_{43}H_{60}N_4O_{10}\cdot H_2O$: C, 63.68; H, 7.71; N, 6.91. Found: C, 63.41; H, 7.66; N, 7.02.

6-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylhexanoyl-L-arginine (**9d**): Colorless crystalline powder (93%). IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1750—1740 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.15—2.33 (5H, m, 3-$OCOCH_2$, C<u>H_2</u>CONH, 15α-H), 2.89 (1H, d, J=13.9 Hz, 17-H), 3.15 (2H, m, C<u>H_2</u>NHC(NH)NH_2, major+minor), 3.81 (3H, s, OCH_3, major), 3.86 (3H, s, OCH_3, minor), 4.16 (1H, m, C<u>H</u>(COOH)NH), 4.69—4.74 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=1.8, 18.6 Hz, 21-H, major), 4.85—4.93 (3H, m, 16-H, 21-H_2, minor), 5.07 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.87 (1H, s, 22-H, major), 6.02 (1H, s, C<u>H</u>-Ph-OCH_3, major), 6.34 (1H, s, C<u>H</u>-Ph-OCH_3, minor), 6.92 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.34 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 807 $(M+H)^+$. *Anal.* Calcd for $C_{44}H_{62}N_4O_{10}\cdot H_2O$: C, 64.06; H, 7.82; N, 6.79. Found: C, 64.21; H, 7.94; N, 6.66.

7-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxy-

carbonylheptanoyl-L-arginine (**9e**): Colorless crystalline powder (86%). IR (KBr) cm^{-1}: 3370 (br, OH, COOH, NH_2), 1750—1730 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.15—2.30 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.88 (1H, d, J=12.8 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 3.80 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.17 (1H, m, CH(COOH)NH), 4.66—4.73 (2H, m, 16-H, 21-H, major), 4.84 (1H, dd, J=1.8, 18.9 Hz, 21-H, major), 4.80—4.96 (3H, m, 16-H, 21-H_2, minor), 5.07 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.85 (1H, s, 22-H, major), 6.01 (1H, s, CH-Ph-OCH_3, major), 6.35 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.7 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.7 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 821 $(M+H)^+$. *Anal.* Calcd for $C_{45}H_{64}N_4O_{10}\cdot H_2O$: C, 64.42; H, 7.93; N, 6.68. Found: C, 64.31; H, 7.66; N, 6.73.

8-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonyloctanoyl-L-arginine (**9f**): Colorless crystalline powder (81%). IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.17—2.44 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.87 (1H, d, J=12.7 Hz, 17-H), 3.17 (2H, m, $CH_2NHC(NH)NH_2$), 3.81 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.14 (1H, m, CH(COOH)NH), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.92 (1H, dd, J=1.8, 18.3 Hz, 21-H, major), 4.85—4.98 (3H, m, 16-H, 21-H_2, minor), 5.07 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, CH-Ph-OCH_3, major), 6.35 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 835 $(M+H)^+$. *Anal.* Calcd for $C_{46}H_{66}N_4O_{10}\cdot H_2O$: C, 64.77; H, 8.03; N, 6.57. Found: C, 64.85; H, 7.73; N, 6.33.

9-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylnonanoyl-L-arginine (**9g**): Colorless crystalline powder (86%). IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1750—1740 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.53—2.67 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.89 (1H, d, J=13.4 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 3.80 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.14 (1H, m, CH(COOH)NH), 4.65—4.72 (2H, m, 16-H, 21-H, major), 4.84 (1H, dd, J=1.8, 18.5 Hz, 21-H, major), 4.80—4.94 (3H, m, 16-H, 21-H_2, minor), 5.07 (1H, br, 3-H), 5.65 (1H, s, 22-H, minor), 5.85 (1H, s, 22-H, major), 6.01 (1H, s, CH-Ph-OCH_3, major), 6.35 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 849 $(M+H)^+$. *Anal.* Calcd for $C_{47}H_{68}N_4O_{10}\cdot H_2O$: C, 65.10; H, 8.14; N, 6.46. Found: C, 64.94; H, 8.05; N, 6.22.

11-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonylundecanoyl-L-arginine (**9h**): Colorless crystalline powder (86%). IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3$, δ: 2.17—2.30 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.89 (1H, d, J=13.4 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 3.81 (3H, s, OCH_3, major), 3.86 (3H, s, OCH_3, minor), 4.15 (1H, m, CH(COOH)NH), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.84 (1H, dd, J=1.8, 18.3 Hz, 21-H, major), 4.83—4.93 (3H, m, 16-H, 21-H_2, minor), 5.09 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, CH-Ph-OCH_3, major), 6.35 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 877 $(M+H)^+$. *Anal.* Calcd for $C_{49}H_{72}N_4O_{10}\cdot H_2O$: C, 65.75; H, 8.33; N, 6.26. Found: C, 65.52; H, 8.11; N, 6.10.

12-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonyldodecanoyl-L-arginine (**9i**): Colorless crystalline powder (95%). IR (KBr) cm^{-1}: 3370 (br, OH, COOH, NH_2), 1750—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.19—2.45 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.92 (1H, d, J=13.4 Hz, 17-H), 3.15 (2H, m, $CH_2NHC(NH)NH_2$), 3.82 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.21 (1H, m, CH(COOH)NH), 4.71—4.75 (2H, m, 16-H, 21-H, major), 4.88 (1H, dd, J=1.8, 18.3 Hz, 21-H, major), 4.82—4.96 (3H, m, 16-H, 21-H_2, minor), 5.10 (1H, br, 3-H), 5.67 (1H, s, 22-H, minor), 5.88 (1H, s, 22-H, major), 6.02 (1H, s, CH-Ph-OCH_3, major), 6.36 (1H, s, CH-Ph-OCH_3, minor), 6.90 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.35 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 891 $(M+H)^+$. *Anal.* Calcd for $C_{50}H_{74}N_4O_{10}\cdot H_2O$: C, 66.05; H, 8.43; N, 6.16. Found: C, 66.01; H, 8.20; N, 6.40.

13-(14β,16β-*O*-(4-Methoxybenzylidene)card-20(22)-enolide-3β-yl)oxycarbonyltridecanoyl-L-arginine (**9j**): Colorless crystalline powder (89%). IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1750—1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.17—2.31 (5H, m, 3-$OCOCH_2$, CH_2CONH, 15α-H), 2.90 (1H, d, J=13.4 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 3.81 (3H, s, OCH_3, major), 3.87 (3H, s, OCH_3, minor), 4.14 (1H, m, CH(COOH)NH), 4.69—4.73 (2H, m, 16-H, 21-H, major), 4.84 (1H, dd, J=1.8, 18.3 Hz, 21-H, major), 4.82—4.93 (3H, m, 16-H, 21-H_2, minor), 5.09 (1H, br, 3-H), 5.66 (1H, s, 22-H, minor), 5.86 (1H, s, 22-H, major), 6.02 (1H, s, CH-Ph-OCH_3, major), 6.35 (1H, s, CH-Ph-OCH_3, minor), 6.88 (2H, d, J=8.6 Hz, Ph-2′-H, 6′-H, major+minor), 7.33 (2H, d, J=8.6 Hz, Ph-3′-H, 5′-H, major+minor). FAB-MS m/z: 905 $(M+H)^+$. *Anal.* Calcd for $C_{51}H_{76}N_4O_{10}\cdot H_2O$: C, 66.35; H, 8.52; N, 6.07. Found: C, 66.09; H, 8.26; N, 6.17.

Cleavage of Methoxybenzilidene Acetal Group of 9a—h Compound **9a** (55.4 mg, 0.0725 mmol) in 80% aqueous AcOH (5 ml) was stirred at room temperature for 12 h. The solvent was removed *in vacuo*, and the residue was chromatographed on SiO_2 ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to give **10a** (37.5 mg, 80%). Compounds **10b**—**j** were similarly obtained from **9b**—**j**, respectively. The physical properties of **10a**—**j** are listed in Table 4.

3-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylpropanoyl-L-arginine (**10a**): IR (KBr) cm^{-1}: 3350 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.48 (2H, m, 3-$OCOCH_2$), 2.55 (2H, t, J=7.3 Hz, CH_2CONH), 2.67 (1H, dd, J=6.7, 14.7 Hz, 15α-H), 3.09 (1H, d, J=7.6 Hz, 17-H), 3.19 (2H, m, $CH_2NHC(NH)NH_2$), 4.26 (1H, m, CH(COOH)NH), 4.61 (1H, t, J=6.6 Hz, 16-H), 5.02 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.09 (1H, br, 3-H), 5.18 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.94 (1H, s, 22-H). FAB-MS m/z: 646 $(M)^+$.

4-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylbutanoyl-L-arginine (**10b**): Yield, 78%. IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.32 (2H, m, 3-$OCOCH_2$), 2.39 (2H, t, J=7.3 Hz, CH_2CONH), 2.60 (1H, dd, J=8.2, 15.2 Hz, 15α-H), 3.12 (1H, d, J=7.3 Hz, 17-H), 3.20 (2H, m, $CH_2NHC(NH)NH_2$), 4.28 (1H, m, CH(COOH)NH), 4.64 (1H, t, J=6.6 Hz, 16-H), 5.02 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.09 (1H, br, 3-H), 5.13 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.94 (1H, s, 22-H). FAB-MS m/z: 660 $(M)^+$.

5-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylpentanoyl-L-arginine (**10c**): Yield, 89%. IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.22 (2H, m, 3-$OCOCH_2$), 2.34 (2H, t, J=6.6 Hz, CH_2CONH), 2.44 (1H, dd, J=6.7, 14.6 Hz, 15α-H), 2.96 (1H, d, J=6.7 Hz, 17-H), 3.20 (2H, m, $CH_2NHC(NH)NH_2$), 4.20 (1H, m, CH(COOH)NH), 4.61 (1H, t, J=6.6 Hz, 16-H), 5.01 (1H, dd, J=1.8, 18.5 Hz, 21-H), 5.08 (1H, br, 3-H), 5.14 (1H, dd, J=1.8, 18.5 Hz, 21-H), 5.94 (1H, s, 22-H). FAB-MS m/z: 674 $(M)^+$.

6-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylhexanoyl-L-arginine (**10d**): Yield, 82%. IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.25 (2H, m, 3-$OCOCH_2$), 2.33 (2H, t, J=7.0 Hz, CH_2CONH), 2.44 (1H, dd, J=6.7, 15.2 Hz, 15α-H), 3.02 (1H, d, J=7.3 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 4.18 (1H, m, CH(COOH)NH), 4.60 (1H, t, J=6.6 Hz, 16-H), 5.01 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.07 (1H, br, 3-H), 5.11 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 688 $(M)^+$.

7-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylheptanoyl-L-arginine (**10e**): Yield, 77%. IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.22 (2H, m, 3-$OCOCH_2$), 2.32 (2H, t, J=7.3 Hz, CH_2CONH), 2.44 (1H, dd, J=6.7, 14.7 Hz, 15α-H), 3.00 (1H, d, J=7.3 Hz, 17-H), 3.18 (2H, m, $CH_2NHC(NH)NH_2$), 4.22 (1H, m, CH(COOH)NH), 4.54 (1H, t, J=6.7 Hz, 16-H), 5.01 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.08 (1H, br, 3-H), 5.12 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 702 $(M)^+$.

8-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonyloctanoyl-L-arginine (**10f**): Yield, 75%. IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.22 (2H, m, 3-$OCOCH_2$), 2.31 (2H, t, J=7.3 Hz, CH_2CONH), 2.46 (1H, dd, J=7.0, 14.6 Hz, 15α-H), 3.02 (1H, d, J=7.3 Hz, 17-H), 3.19 (2H, m, $CH_2NHC(NH)NH_2$), 4.24 (1H, m, CH(COOH)NH), 4.56 (1H, t, J=6.7 Hz, 16-H), 5.02 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.08 (1H, br, 3-H), 5.13 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 716 $(M)^+$.

9-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylnonanoyl-L-arginine (**10g**): Yield, 76%. IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2, 1750—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.21 (2H, m, 3-$OCOCH_2$), 2.32 (2H, t, J=7.3 Hz, CH_2CONH), 2.44 (1H, dd,

J=7.0, 14.7 Hz, 15α-H), 3.01 (1H, d, J=7.0 Hz, 17-H), 3.18 (2H, m, C$\underline{H}_2$NHC(NH)NH_2), 4.22 (1H, m, C$\underline{H}$(COOH)NH), 4.54 (1H, t, J=6.6 Hz, 16-H), 4.99 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.08 (1H, br, 3-H), 5.12 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 730 $(M)^+$.

11-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonylundecanoyl-L-arginine (**10h**): Yield, 86%. IR (KBr) cm^{-1}: 3380 (br, OH, COOH, NH_2), 1740—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.21 (2H, m, 3-OCOCH_2), 2.32 (2H, t, J=7.3 Hz, C$\underline{H}_2$CONH), 2.44 (1H, dd, J=7.0, 14.7 Hz, 15α-H), 3.01 (1H, d, J=7.3 Hz, 17-H), 3.18 (2H, m, C$\underline{H}_2$NHC(NH)NH_2), 4.23 (1H, m, C$\underline{H}$(COOH)NH), 4.54 (1H, t, J=6.2 Hz, 16-H), 5.01 (1H, dd, J=1.5, 18.3 Hz, 21-H), 5.08 (1H, br, 3-H), 5.13 (1H, dd, J=1.5, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 758 $(M)^+$.

12-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonyldodecanoyl-L-arginine (**10i**): Yield, 91%. IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1730—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.21 (2H, m, 3-OCOCH_2), 2.36 (2H, t, J=7.3 Hz, C$\underline{H}_2$CONH), 2.46 (1H, dd, J=6.6, 14.7 Hz, 15α-H), 3.03 (1H, d, J=7.3 Hz, 17-H), 3.21 (2H, m, C$\underline{H}_2$NHC(NH)NH_2), 4.25 (1H, m, C$\underline{H}$(COOH)NH), 4.55 (1H, t, J=6.6 Hz, 16-H), 5.02 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.09 (1H, br, 3-H), 5.18 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 772 $(M)^+$.

13-(14β,16β-Dihydroxycard-20(22)-enolide-3β-yl)oxycarbonyltridecanoyl-L-arginine (**10j**): Yield, 90%. IR (KBr) cm^{-1}: 3400 (br, OH, COOH, NH_2), 1730—1720 (C=O). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 2.21 (2H, m, 3-OCOCH_2), 2.32 (2H, t, J=7.3 Hz, C$\underline{H}_2$CONH), 2.43 (1H, dd, J=6.7, 14.7 Hz, 15α-H), 2.99 (1H, d, J=7.3 Hz, 17-H), 3.18 (2H, m, C$\underline{H}_2$NHC(NH)NH_2), 4.23 (1H, m, C$\underline{H}$(COOH)NH), 4.53 (1H, t, J=6.6 Hz, 16-H), 4.99 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.09 (1H, br, 3-H), 5.18 (1H, dd, J=1.8, 18.3 Hz, 21-H), 5.95 (1H, s, 22-H). FAB-MS m/z: 786 $(M)^+$.

Measurement of Cardiotonic Activities The cardiotonic activities (pIC_{50} and pD_2 values) of test compounds were examined by using an Na^+,K^+-ATPase preparation from dog kidney (Sigma Co., Ltd.) and an isolated guinea-pig papillary muscle preparation, respectively. The measurements were performed as described before.[5,9]

Measurement of the Activity on Arterial Smooth Muscle The effects on arterial smooth muscle were examined by the same method as described before.[5,10]

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