Cardiac Glycosides from *Antiaris toxicaria* with Potent Cardiotonic Activity

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An ethanolic extract of *Antiaris toxicaria* trunk bark showed potent in vitro cardiotonic effect on isolated guinea pig atria. Bioassay-guided fractionation of the extract led to identification of nine new cardiac glycosides (**1**−**9**, named antiarosides A−I), antiarotoxinin A (**10**), and 18 known compounds. Their structures were established using MS and NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments. The ability of these cardiotonic compounds to produce positive inotropic action and their safety indexes were examined in comparison with those of ouabain, a classical inhibitor of Na^+/K^+-ATPase. Malayoside (**23**) was nearly equipotent and had a similar safety index to ouabain in guinea pig atria. However, the maximal positive inotropic effect and safety index of **23** in papillary muscle were better than those of ouabain. An electrophysiological recording showed that **23** inhibited the sodium pump current in a concentration-dependent manner.

Cardenolides are a group of C_{23} steroids produced in nature by several plant families and some species of toads. Because a major site of their biological action is the heart, cardenolide glycosides are also known as cardiac glycosides. These compounds share common features of a steroidal aglycone linked at the 3β-OH group to one or more sugar moieties. Some cardiac glycosides are highly toxic to humans and animals. Despite their toxicity, certain glycosides have therapeutic effects and, at appropriate doses, have been used in the treatment of congestive heart failure. The only receptor for these compounds is the integral membrane protein Na^+/K^+-ATPase. Cardiac glycosides inhibit Na^+/K^+-ATPase, resulting in a positive inotropic effect at therapeutic doses, but also in cardiac arrhythmias and death at toxic doses. Apart from their very widely known cardiotonic effects, cardiac glycosides may also inhibit cancer cell replication. Cardiac glycosides also act directly on the gastrointestinal tract, causing hemorrhagic enteritis and diarrhea.

Antiaris toxicaria (Pers.) Lesch. (Moraceae), commonly known as the upas tree, is a well-known toxic plant that is widely distributed throughout Malaysian forests. The latex of *A. toxicaria*, called "Jianxiefenghou" in Chinese, has an unwarranted reputation for killing people who fall asleep beneath it. It has been known for centuries that most poisoned darts used by indigenous people of Southeast Asia are prepared by concentration of latex harvested from *A. toxicaria*. Prey wounded by such an arrow can rarely move more than 100 m. These poisons act as powerful muscle relaxants to paralyze the prey, but have no effect when the meat is eaten.[1] Bisset reported that animals shot with poisoned darts "died with tetanic convulsions", indicating that *A. toxicaria*-derived poisons function through the bloodstream.[2] The notoriety of these materials prompted investigations of their constituents, and they were found to be a good source of cardenolide cardiac glycosides.[3−7] The active principles were studied by Robinson and Ling, who observed cardiac irregularities and death when extracts of *A. toxicaria* were injected into cats.[8] Fujimoto *et al.* first found that cardiac glycosides inhibited the activity of Na^+/K^+-ATPase.[9]

In the course of a drug-screening project on medicinal plants, an extract from the trunk bark of *A. toxicaria* was found to have a strong cardiotonic effect. Bioassay-guided fractionation led to the isolation and characterization of 28 cardiac glycosides/aglycones, including new compounds **1**−**10**, designated as antiarosides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), H (**8**), and I (**9**) and antiarotoxinin A (**10**). This study also evaluated the positive inotropic effect and safety index of these compounds in guinea pig heart muscle. The goals were to (a) find an effective and safe inotropic drug for improving hemodynamics in patients with heart failure and (b) establish structure−activity relationships of cardiac glycosides. Herein, we report the maximal positive inotropic effect and safety index of new compounds **1**−**10**, as well as of known compounds **11**−**14** and **16**−**28**, in guinea pig heart muscle. The positive control was ouabain, a classical inhibitor of Na^+/K^+-ATPase.

Results and Discussion

Fresh trunk bark of *A. toxicaria* was extracted with ethanol. The residue obtained after evaporation of the ethanol extract was partitioned between $CHCl_3$, *n*-BuOH, and H_2O. The $CHCl_3$-, *n*-BuOH-, and H_2O-soluble fractions were concentrated, and the extracts were individually subjected to column chromatography (CC) over silica gel and Diaion HP-20, respectively. The subfractions obtained were examined by H_2SO_4 test solution spray on TLC for cardiac glycosides, which appear as green spots. The enriched cardiac glycoside fractions were subjected to a series of column chromatographic steps (silica gel, semipreparative reversed-phase HPLC, Sephadex LH-20) in order to obtain pure cardenolides **1**−**28**, which were characterized by analysis of their spectroscopic data.

Compound **1** had the molecular formula $C_{29}H_{44}O_9$, as established from HRFABMS ($[M + Na]^+$ *m*/*z* 559.3063) and by the presence of 29 signals in the ^{13}C NMR spectrum. The NMR signals were due to three methyl, 10 methylene, 10 methine, and six quaternary carbons, as determined using DEPT 135 spectroscopy (Table 1). Compound **1** showed a UV absorption maximum at 213 nm and IR absorption at 1738 cm^{-1} (γ-lactonic carbonyl), which were

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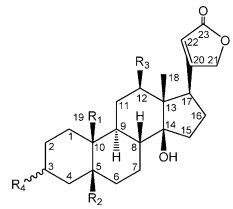
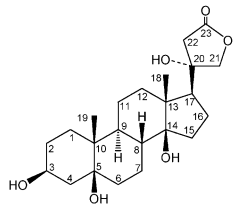


Compd	R_1	R_2	R_3	R_4
1	CH_3	OH	H	*β*-*O*-*β*-D-antiarose
2	CH_2OH	H	H	*β*-*O*-*α*-L-rhamnosyl-(4→1)-*β*-D-glucose
3	CHO	OH	H	*α*-*O*-*α*-L-rhamnose
4	CHO	OH	H	*α*-*O*-*α*-L-rhamnosyl-(4→1)-*β*-D-glucose
5	COOH	H	H	*β*-*O*-*α*-L-rhamnose
6	COOglc	H	H	*β*-*O*-*α*-L-rhamnose
7	COOH	OH	OH	*β*-*O*-*α*-L-rhamnose
8	COOH	OH	OH	*β*-*O*-*β*-D-antiarose
9	H	OH	H	*β*-*O*-*α*-L-rhamnose
23	CHO	H	H	*β*-*O*-*α*-L-rhamnose



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indicative of a butenolactone system.[10] The ^{1}H NMR spectrum of **1** (Table 2) showed characteristic signals of a butenolactone ring at *δ* 5.01 and 5.28 (each 1H, dd, *J* = 18.0, 1.3 Hz, H-21a and -b) and 6.12 (s, H-22) and methyl singlets at *δ* 1.03 and 1.08 (each 3H, s, H-18 and -19), indicating **1** to be a cardenolide with a C-19 methyl group. A doublet anomeric proton at *δ* 5.37 (*J* = 8.1 Hz, H-1′), four oxymethine protons between *δ* 4.10 and 4.71, and a methyl signal at *δ* 1.56 (3H, d, *J* = 6.4 Hz), together with the fragment ion at *m*/*z* 373 in the FABMS, pointed to the presence of a *β*-linked deoxyhexose unit (Table 1). The deoxyhexose was concluded to be *β*-antiarose on the basis of ^{1}H and ^{13}C NMR data (Tables 1 and 2) and analyses of ROESY, COSY, and HMQC experiments.[7,11] The proton resonating at *δ* 4.45 (H-2′), a double doublet (*J* = 8.1, 2.9 Hz), indicated an axial−axial relationship to H-1′ and an axial−equatorial relationship to H-3′ (d, *J* = 2.9 Hz). On the basis of the strong NOE effects between H-1′ and H-5′, both protons are in axial positions. Furthermore, the measured $J_{C1'-H1'}$ (157.6 Hz) for the anomeric axial proton is consistent with *β*-D chemistry of the sugar moiety.[12] Thus, on the basis of the above data, the sugar moiety was elucidated as *β*-D-antiarose. The aglycone was identified as periplogenin[13] by analyses of COSY, HMQC, HMBC, and ROESY experiments and comparison to literature data. The sugar unit was placed at C-3 on the basis of the glycosylation shifts of C-2 (*δ* 26.9), C-3 (*δ* 74.4), and C-4 (*δ* 34.5) and HMBC correlation between H-1′ of the antiarose unit and C-3 of the aglycone moiety. The *β*-orientation of antiarose at C-3 was deduced from the $W_{1/2}$ constant of H-3 (br s, $W_{1/2}$ = 10.7 Hz). Thus, **1** was identified as periplogenin 3-*O*-*β*-antiaropyranoside and was named antiaroside A.

The HRFAB mass spectrum of **2** showed a molecular ion-related $[M + K]^+$ peak at *m*/*z* 737.8260, corresponding to the molecular formula $C_{35}H_{54}O_{14}$. The ^{1}H and ^{13}C NMR data (Tables 1and 2) and fragment ions at *m*/*z* 519 and 356 in the FABMS indicated **2** was a cardenolide disaccharide with two hexose sugar units. Analyses of COSY, HMQC, and HMBC spectra indicated that the sugars were α-rhamnose and *β*-glucose with a (4−1) linkage. The downfield shift of C-4′ of the rhamnose unit to *δ* 85.5, and C-1″

Table 1. ^{13}C NMR Data of Compounds Isolated from *A. toxicaria*[a]

carbon	**1**	**2**	**3**[b]	**4**	**5**	**6**	**7**[b]	**8**[b]	**9**	**10**
1	26.2	24.9	25.8	25.8	21.8	21.8	21.9	21.8	22.2	25.9
2	26.9	26.9	30.7	30.7	26.8	26.3	26.5	27.0	29.2	28.6
3	74.4	72.7	72.5	72.6	71.2	71.6	72.9	73.4	71.6	67.8
4	34.5	27.4	35.5	35.5	29.1	26.5	36.2	36.0	31.9	35.9
5	73.8	30.2	71.3	72.7	32.8	32.4	73.9	73.7	72.2	74.5
6	36.2	30.5	42.8	42.9	29.7	30.1	36.6	37.5	32.0	37.9
7	24.6	21.8	22.3	22.3	26.5	22.2	24.5	24.8	27.1	24.4
8	41.1	42.0	42.9	42.9	42.3	41.9	40.4	40.4	41.5	39.7
9	39.4	35.9	41.9	41.9	35.7	36.0	36.3	36.4	38.4	39.1
10	41.3	39.4	56.2	56.2	49.7	50.4	54.9	55.1	37.4	41.4
11	22.0	22.0	22.0	22.0	22.5	28.7	33.3	33.0	20.9	21.4
12	40.1	40.5	39.6	39.7	40.2	40.1	74.6	74.7	39.8	40.4
13	50.0	50.3	50.0	50.0	50.5	50.0	57.0	57.0	50.2	49.2
14	84.8	85.0	84.4	84.4	84.9	84.9	85.3	85.3	84.5	85.1
15	33.3	33.1	32.8	32.8	33.0	32.9	32.9	33.2	33.1	44.2
16	27.4	27.2	27.2	27.2	27.4	27.3	28.0	28.1	27.4	23.7
17	51.5	51.6	51.4	51.4	51.5	51.4	46.6	46.6	51.5	59.0
18	16.2	16.3	16.0	16.0	16.4	16.2	10.5	10.5	16.2	16.6
19	17.2	65.6	208.3	208.3	179.5	175.7	176.9	176.8		17.4
20	175.7	175.9	175.7	175.6	176.4	176.0	176.7	177.2	176.2	79.5
21	73.7	73.8	73.7	73.7	73.9	73.7	74.1	74.1	73.8	81.1
22	117.8	117.7	117.8	117.8	117.7	117.7	117.5	117.5	117.6	32.3
23	174.3	174.5	174.4	174.4	174.7	174.5	174.8	174.9	174.6	176.3
1′	100.0	99.8	99.9	99.7	99.7	100.0	100.5	99.8	99.9	
2′	69.5	72.4	72.8	71.7	73.0	72.8	73.0	69.6	72.9	
3′	73.7	72.8	72.8	72.3	72.9	72.8	72.6	73.7	72.9	
4′	73.3	85.5	74.2	85.1	74.1	74.2	73.9	73.7	74.2	
5′	69.9	68.4	69.9	68.2	70.3	70.0	70.7	70.0	70.2	
6′	16.9	18.5	18.6	18.4	18.8	18.6	18.7	17.1	18.7	
1″		106.9		106.7		95.7				
2″		76.5		76.4		74.1				
3″		78.6		78.6		79.1				
4″		71.7		21.3		71.3				
5″		78.4		78.4		79.4				
6″		62.8		62.8		62.5				

[a] *δ* values in pyridine-d_5 (100 MHz). [b] *δ* values in pyridine-d_5 (75 MHz).

Table 2. ^{1}H NMR Data of Compounds Isolated from *A. toxicaria*[a]

proton	**1**	**2**
1a/b	1.44 (m)/2.19 (dd, 14.2, 2.9)	1.66 (br t, 4.1)/2.33 (ddd, 13.5, 13.5, 3.1)
2a/b	1.79 (m)/2.02 (br d, 13.0)	1.72 (m)/1.89 (m)
3	4.51 (br s, 10.7)[b]	4.15 (br s, 13.6)[b]
4a/b	1.88 (m)/2.15 (dd, 15.2, 2.9)	1.25 (br d, 12.8)/2.01 (m)
5		2.55 (br d, 13.3)
6a/b	1.54 (m)/1.95 (m)	1.57 (m)/1.89 (m)
7a/b	1.33 (m)/2.23 (br d, 11.7)	1.43 (m)/2.14 (m)
8	1.83 (m)	2.01 (m)
9	1.67 (br t, 11.4)	1.89 (m)
10		
11a/b	1.38 (br d, 10.0)/1.44 (m)	1.43 (m)
12a/b	1.44 (m)	1.48 (m)
15a/b	1.88 (m)/2.08 (m)	1.89 (m)/2.17 (br dd, 10.4, 10.4)
16a/b	1.95 (m)/2.08 (m)	2.01 (m)/2.14 (m)
17	2.81 (dd, 8.8, 5.0)	2.81 (dd, 9.0, 5.1)
18	1.03 (s)	1.04 (s)
19a/b	1.08 (s)	3.77 (dd, 10.9, 5.5)/4.07 (d, 10.9)
21a/b	5.01 (dd, 18.0, 1.3)/5.28 (dd, 18.0, 1.3)	5.01 (d, 18.0)/5.29 (d, 18.0)
22	6.12 (s)	6.12 (s)
1′	5.37 (d, 8.1)	5.37 (br s)
2′	4.45 (dd, 8.1, 2.9)	4.49 (br s)
3′	4.71 (d, 2.9)	4.62 (dd, 9.1, 2.1)
4′	4.10 (br s)	4.34 (d, 9.1)
5′	4.58 (q, 6.4)	4.29 (dq, 9.1, 6.0)
6′	1.56 (d, 6.4)	1.70 (d, 6.0)
1″		5.18 (d, 7.7)
2″		4.05 (d, 7.7)
3″		4.18 (d, 9.1)
4″		4.21 (d, 9.1)
5″		3.79 (m)
6″a/b		4.39 (m)/ 4.43 (m)

proton	**3**	**4**
1a/b	2.07 (m)/2.34 (dd, 10.1, 4.2)	2.03 (m)/2.32 (m)
2a/b	1.61 (m)/2.29 (m)	1.57 (ddd, 13.4, 13.4, 4.3)/2.28 (m)
3	4.66 (dddd, 11.2, 11.2, 5.0, 4.3)	4.60 (dddd, 10.4, 10.4, 5.1, 5.1)
4a/b	1.78 (br d, 13.6)/2.42 (dd, 13.6, 4.3)	1.72 (m)/2.39 (dd, 13.5, 5.1)
5		
6a/b	1.68 (m)/2.22 (m)	1.63 (m)/1.77 (m)
7a/b	1.68 (m)/2.29 (m)	2.11 (br dd, 8.0, 3.7)/2.28 (m)
8	2.22 (m)	2.16 (ddd, 13.7, 13.7, 4.3)
9	1.40 (m)	2.23 (m)
10		
11a/b	1.40 (m)/1.68 (m)	1.43 (m)/1.68 (br d, 10.2)
12a/b	1.40 (m)	1.41 (m)/ 1.27 (dd, 14.3, 3.1)
15a/b	1.29 (dd, 13.6, 3.0)/1.83 (m)	1.41 (m)/ 1.85 (m)
16a/b	2.13 (m)/ 1.91 (m)	2.05 (ddd, 13.5, 3.5, 3.4)/1.90 (ddd, 13.5, 9.0, 4.8)
17	2.02 (dd, 8.8, 4.8)	1.99 (m)
18	2.73 (dd, 9.3, 4.8)	2.74 (ddd, 9.0, 4.8)
19a/b	0.95 (s)/ 10.12 (s)	0.96 (s)/ 0.13 (s)
21a/b	4.98 (dd, 18.0, 1.3)/5.25 (dd, 18.0, 1.3)	4.97 (dd, 18.0, 1.4)/5.25 (dd, 18.0, 1.4)
22	6.09 (s)	6.09 (s)
1′	5.36 (br s)	5.30 (br s)
2′	4.43 (br s)	4.40 (br s)
3′	4.44 (dd, 8.6, 3.3)	4.54 (d, 10.1)
4′	4.21 (m)	4.32 (dd, 10.1, 9.3)
5′	4.26 (dq, 8.6, 5.8)	4.18 (m)
6′	1.59 (d, 5.8)	1.63 (d, 6.1)
1″		5.20 (d, 7.7)
2″		4.08 (dd, 8.1, 7.7)
3″		4.18 (m)
4″		4.18 (m)
5″		3.78 (ddd, 8.4, 4.5, 4.5)
6″a/b		4.34 (m)/4.42 (m)

proton	**5**	**6**
1a/b	1.60 (ddd, 12.6, 12.6, 3.1)/2.51 (m)	1.55 (ddd, 14.0, 10.0, 2.6)/2.37 (ddd, 12.9, 12.9, 3.8)
2a/b	1.99 (m)/2.54 (m)	1.64 (m)/1.91 (m)
3	4.52 (br s, 12.4)[b]	4.17 (br s, 10.7)[b]
4a/b	1.38 (m)/1.99 (m)	2.00 (m)/2.64 (dd, 14.0, 3.3)
5	2.92 (br d, 12.6)	2.88 (br d, 11.9)
6a/b	1.70 (m)/1.97 (m)	1.64 (m)/1.86 (m)
7a/b	1.67 (m)/1.91 (m)	1.41 (m)/2.07 (m)
8	2.59 (m)	2.52 (ddd, 11.7, 11.7, 2.4)
9	1.91 (m)	1.91 (m)

Table 2 Continued

proton	**5**	**6**
10		
11a/b	1.42 (dd, 129, 12.9)/2.17 (m)	1.20 (m)/2.00 (m)
12a/b	1.48 (m)	1.41 (m)
15a/b	1.87 (m)/2.11 (m)	1.86 (m)/2.12 (m)
16a/b	2.06 (m)	2.00 (m)/2.12 (m)
17	2.84 (dd, 8.3, 4.2)	2.79 (br dd, 8.4, 5.2)
18	1.20 (s)	1.17 (s)
19		
21a/b	5.06 (d, 18.0)/5.35 (d, 18.0)	5.00 (d, 18.1)/5.28 (d, 18.1)
22	6.14 (s)	6.10 (s)
1′	5.48 (br s)	5.39 (br s)
2′	4.57 (br s)	4.46 (m)
3′	4.52 (d, 8.2)	4.46 (m)
4′	4.32 (m)	4.22 (m)
5′	4.32 (m)	4.30 (dq, 6.0, 6.0)
6′	1.67 (d, 5.1)	1.65 (d, 6.0)
1″		6.29 (d, 8.0)
2″		4.13 (dd, 8.0, 7.7)
3″		4.22 (m)
4″		4.22 (m)
5″		4.01 (br dd, 7.1, 4.0)
6″a/b		4.31 (dd, 5.9, 3.4)/4.38 (d, 11.3)
proton	**7**[c]	**8**[c]
1a/b	2.27 (br d, 14.7)/ 2.97 (br d, 14.7)	2.28 (d, 16.4)/3.16 (dd, 14.7, 14.7)
2a/b	1.78 (br d, 14.1)/ 1.98 (m)	1.87 (m)/2.16 (m)
3	4.35 (br s, 9.3)[b]	4.51 (br s, 12.1)[b]
4a/b	1.87 (br d, 14.5)/ 1.98 (m)	2.00 (m)/2.16 (m)
5		
6a/b	1.65 (m)/ 3.13 (br d, 13.1)	1.69 (br d, 12.6)/3.11 (m)
7a/b	1.48 (m)/ 2.49 (d, 12.7)	1.47 (br d, 13.4)/2.48 (br d, 11.9)
8	3.06 (br d, 10.1)	3.04 (m)
9	2.19 (m)	2.00 (m)
10		
11a/b	1.98 (m)/2.39 (dd, 7.4, 3.7)	2.00 (m)
12a/b	−/3.81 (br d, 10.8)	−/3.77 (br d, 6.9)
15a/b	1.98 (m)/1.98 (m)	2.00 (m)/ 2.35 (m)
16a	2.16 (m)	2.16 (m)
17	3.75 (t, 7.5)	3.75 (t, 7.9)
18	1.26 (s)	1.26 (s)
19		
21a/b	5.13 (d, 18.1)/5.29 (d, 18.1)	5.12 (d, 18.1)/5.28 (d, 18.1)
22	6.24 (s)	6.25 (s)
1′	5.54 (br s)	5.44 (d, 8.1)
2′	4.53 (br s)	4.49 (dd, 8.1, 2.6)
3′	4.46 (br d, 4.7)	4.76 (br s)
4′	4.29 (m)	4.14 (d, 3.0)
5′	4.27 (br q, 3.7)	4.62 (q, 6.2)
6′	1.65 (d, 3.7)	1.56 (d, 6.2)
1″		
2″		
3″		
4″		
5″		
6″a/b		
proton	**9**	**10**
1a/b	1.38 (br d, 13.8)/2.19 (br d, 17.0)	1.48 (m)/2.24 (m)
2a/b	1.65 (m)/ 2.00 (m)	1.82 (m)
3	4.22 (br s, 9.1)[b]	4.44 (br s, 12.8)[b]
4a/b	1.79 (m)/2.10 (m)	1.78 (d, 10.1)/2.29 (d, 14.6)
6a/b	1.79 (m)/2.10 (m)	1.62 (br d, 13.0)/1.96 (dt, 9.1, 4.0)
7a/b	1.26 (m)/2.41 (br d, 7.9)	2.24 (m)
8	2.43 (br d, 11.5)	1.97 (dt, 9.1, 4.0)
9	2.31 (br d, 12.5)	1.70 (br t, 10.7)
10	1.83 (d, 12.5)	
11a/b	1.06 (br d, 12.0)/1.90 (m)	1.45 (ddd, 10.6, 9.6, 4.0)
12a/b	1.44 (m)	1.36 (dt, 13.3, 10.6)/1.48 (dt, 13.3, 9.6)
15a/b	1.90 (m)/2.10 (m)	1.84 (m)/2.04 (m)
16	2.00 (m)	2.04 (m)
17	2.10 (m)	2.14 (dd, 8.2, 5.7)
18	2.79 (br d, 7.8)	1.27 (s)
19	1.06 (s)	1.17 (s)
21a/b	5.03 (d, 18.1)/5.33 (d, 18.1)	4.40 (d, 9.4)/4.78 (d, 9.4)
22	6.12 (s)	2.82 (d, 16.8)/2.86 (d, 16.8)

Table 2 Continued

proton	9	10
1′	5.44 (br s)	
2′	4.50 (m)	
3′	4.50 (m)	
4′	4.29 (m)	
5′	4.29 (m)	
6′	1.65 (d, 3.8)	
1″		
2″		
3″		
4″		
5″		
6″a/b		

[a] δ values in pyridine-d_5 (400 MHz); coupling constants in Hz are given in parentheses. [b] $W_{1/2}$ (Hz): width of half-peak height. [c] δ values in pyridine-d_5 (300 MHz); coupling constants in Hz are given in parentheses.

of the glucose unit at δ 106.9, and an HMBC correlation between H-4′ and C-1″ confirmed the (4−1) linkage between them. The ^{13}C NMR spectrum (Table 1), combined with DEPT 135, HMQC, and HMBC experiments of **2** indicated that the aglycone was cannogenol (**15**), which was isolated from the $CHCl_3$-soluble fraction. Location of the sugar unit at C-3 was suggested by the downfield shift of C-3 from δ 66.1 in **15** to δ 72.7 in **2** and a HMBC correlation between H-1′ and C-3. The β-orientation of the C-3 disaccharide unit was deduced from the $W_{1/2}$ constant of H-3 (br s, $W_{1/2}$ = 13.6 Hz). Compound **2** was thus assigned as 3β-[(*O*-β-glucopyranosyl(1−4)-α-rhamnopyranosyl)oxy]cannogenol, and it was named antiaroside B.

Compound **3** showed a pseudo molecular ion peak at *m*/*z* 551.2855 in its HRFABMS and had the same molecular formula as convallatoxin (**17**), $C_{29}H_{42}O_{10}$. UV, IR, ^{1}H and ^{13}C NMR, and MS spectroscopic analyses indicated that **3** was a stereoisomer of convallatoxin (**17**). Downfield shifts of C-1, C-2, and C-6 from δ 18.7, 25.4, and 36.9 in **17** to δ 25.8, 26.0, and 42.8 in **3** indicated that the orientation of C-3 was different from that of **17**. The α-orientation of C-3 was deduced from the coupling constant values of H-3 (dddd, J = 11.2, 11.2, 5.0, 5.0 Hz). This assignment was supported by downfield shifts of H-2b, H-3, and H-4 from δ 2.00 (m), 4.32 (br s, $W_{1/2}$ = 8.3 Hz), 2.13 (d, J = 15.3 Hz), and 1.72 (m) in **17** to δ 2.29 (m), 4.66 (dddd, J = 11.2, 11.2, 5.0, 4.3 Hz), 2.42 (dd, J = 13.6, 4.3 Hz), and 1.78 (br d, J = 13.6 Hz) in **3**. HMBC correlation of H-1′ with C-3 [δ_C 72.5/δ_H 5.36 (br s)] and a NOE correlation between H-3 and H-1′ inferred that the rhamnose unit was linked to C-3. Hence, structure **3** was established for antiaroside C.

Compound **4** was assigned the molecular formula $C_{35}H_{52}O_{15}$ by HRFABMS. Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **3** showed that the two structures were very similar, except for one additional sugar unit in **4**. On the basis of its larger $[M + K]^+$ ion at 751, 162 mass units more than that of **3**, and appropriate sugar proton and carbon signals in the NMR spectra, **4** has one glucosyl unit in addition to a rhamnosyl moiety. The H-1″ signal appeared at δ 5.20 and showed HMBC correlation with the downfield shifted C-4′ (δ 85.1), as well as a NOE with H-4′. These data determined the interglycosydic linkage of the two sugar moieties as α-rhamnosyl (4′−1′)-β-glucoside. A HMBC correlation between H-1′ and C-3 (δ 72.5) suggested that the sugar unit was attached at C-3, and α-orientation was deduced from the coupling type and constant values of H-3 (δ 4.60, dddd, J = 10.4, 10.4, 5.1, 5.1 Hz). Thus, the structure of **4** was deduced as 4′-*O*-β-glucopyranosyl antiaroside C and was named antiaroside D.

Compound **5** had the same molecular formula as **17** ($C_{29}H_{42}O_{10}$). Comparison of the ^{1}H/^{13}C NMR spectra of **5** and **17** showed that the two structures were very similar, except for the absence of signals for both an aldehyde and one oxygenated carbon in the former. A strong carbonyl absorption in the IR spectrum at 1738 cm^{-1} and a carbon signal at δ 179.5 in the ^{13}C NMR spectrum suggested that a carboxylic acid rather than aldehyde group was present at C-19. A proton signal at δ 2.92 (br d, J = 12.6 Hz) in the ^{1}H NMR was assignable to H-5, since it coupled with H-4 and -6 in the COSY spectrum. The absence of a carbon signal at δ 73.9 (C-5 in **17**) and the presence of a carbon signal at δ 32.8 (C-5 in **5**) suggested that the OH group on C-5 in **17** was not present in **5**. This postulate was supported by upfield shifts of C-4 and C-6 from δ 35.5 to δ 29.1 and δ 36.9 to δ 29.7, together with HMBC correlations of H-3, -4b, -6b, and -7b to C-5. In a ROESY experiment, a correlation between H-5 and H-1b (δ 2.51) determined the β-orientation of H-5. An anomeric proton signal at δ 5.48 (br s) and carbon signals at δ 99.7, 73.0, 72.9, 74.1, 70.3, and 18.8 indicated the presence of a rhamnosyl moiety. The HMBC correlation between H-3 and C-1′ placed the rhamnosyl unit on C-3, and a $W_{1/2}$ coupling constant of 12.4 Hz for H-3 indicated the α-orientation. Therefore, the structure **5** was established for antiaroside E.

Compound **6** ($C_{35}H_{52}O_{15}$) had fragment ions at *m*/*z* 550 and 534 in the FABMS, and two anomeric signals in the ^{1}H/^{13}C NMR spectra indicated that **6** was a diglycoside with β-glucopyranose and α-rhamnose sugar units. The ^{1}H and ^{13}C NMR data of **6** were very close to those of **5**, except for the added signals of a glucopyranose moiety and the position of C-19 (Tables 1 and 2). An anomeric proton signal at δ 6.29 (d, J = 8.0 Hz) in the ^{1}H NMR spectrum and signals at δ 95.7, 74.1, 79.1, 71.3, 79.4, and 62.5 in the ^{13}C NMR spectrum suggested the presence of β-glucose. The upfield shift of C-19 from δ 179.5 to δ 175.7, the downfield shift of the anomeric proton to δ 6.29, and a ^3J HMBC correlation from H-1″ to C-19 suggested that the glucose unit was attached to C-19. Thus, the structure of **6** was determined as 19-*O*-β-glucopyranosyl antiaroside E, and it was named antiaroside F.

Compound **7** ($C_{29}H_{42}O_{12}$) was 16 mass units larger than β-antiarin (**28**),[13] isolated from the $CHCl_3$-soluble fraction. The ^{1}H and ^{13}C NMR spectra of **7** were quite similar to those of **28**, except for absence of the aldehyde signal in **7**. Thus, **7** was likely a 19-*nor*-β-antiarin derivative. The major differences were that the proton signal at δ 10.37 (s) and the carbonyl signal at δ 208.5 in **28** disappeared in **7**, and instead one carboxyl carbon signal appeared at δ 176.9. Thus, the aldehyde group of **28** was replaced by a carboxylic acid group in **7**. This conclusion was supported by a strong IR absorption at 1726 cm^{-1}. The β-orientations of OH groups at C-3 and -12 were deduced from the coupling constants of H-3 (δ 4.35, br s, $W_{1/2}$ = 9.3 Hz) and H-12 (br d, J = 10.8 Hz). On the basis of the above data, the structure of **7** was established, and it was named antiaroside G.

Compound **8** had the same molecular formula as that of **7** ($C_{29}H_{42}O_{12}$). The UV, IR, and NMR data strongly resembled those of **7**, consistent with a general structure containing a central cardenolide moiety trioxygenated at C-3, C-5, and C-12 and a carboxylic acid group in the 19-position. The sole significant differences observed were in signals of the glycosidic part of the

molecules (Tables 1 and 2). The sugar proton signals of **8** indicated the presence of a β-antiarosyl moiety. These data were in agreement with the replacement of the rhamnosyl unit in **7** by an antiarosyl unit in **8**. The β-orientation of C-3 was deduced by the $W_{1/2}$ of H-3 (br s, 12.1 Hz). Thus, the structure of **8** was assigned, and it was named antiaroside H.

A molecular formula of $C_{28}H_{42}O_9$ was deduced for compound **9**, 14 mass units less than that of periplorhamnoside (**11**), which was isolated from the same extract. Comparison of its ^{1}H and ^{13}C NMR data with those of **11** showed that they were similar except for the absence of the C-19 methyl group, the presence of one methine (−CH) at δ 1.83 (d, 12.5), and an upfield shift of C-10 from δ 41.2 to δ 37.4 in **9**. Thus, **9** was determined as demethylperiplorhamnoside, and it was named antiaroside I.

Compound **10** ($C_{23}H_{36}O_6$) had 23 signals in the ^{13}C NMR spectrum corresponding to two methyl, 11 methylene, four methine, and six quaternary carbon atoms. The ^{1}H and ^{13}C NMR spectra of **10** displayed signals characteristic of the steroid core of a cardenolide. However, the absence of typical signals for the olefinic group of the butenolactone ring and the downfield resonance of C-23 to 176.3 suggested that the carbonyl group of the five-membered lactone in **10** was not conjugated with a double bond. This was confirmed by the carbonyl absorption at 1761 cm^{-1} in the IR spectrum, the presence of two methylene groups at δ_C 81.1/δ_H 4.78 and 4.40 (each 1H, d, J = 16.8 Hz, H-21) and δ_C 32.3/δ_H 2.86 and 2.82 (each 1H, d, J = 9.4 Hz, H-22), and the absence of any significant UV absorption. Three OH groups were on the steroid skeleton (C-3, C-5, C-14), and a fourth OH group was placed at C-20; this latter carbon resonated at δ 79.5 and no extra oxygenated signal, other than aforementioned, was observed in the spectrum of **10**. HMBC correlations from H-16, H-21b, and H-22 to C-20 and from H-21b to C-22 confirmed the OH group at C-20. The orientation of the C-3 OH was also determined by the $W_{1/2}$ of H-3 (br s, 12.1 Hz). The above analysis established the structure of **10** as shown, and the compound was named antiarotoxinin A.

Periplorhamnoside (**11**),[13] cheiranthoside VII (**12**),[11] strophanthidol (**13**),[14] convallatoxol (**14**),[5] cannogenol (**15**),[15] strophanthidin (**16**),[13] convallatoxin (**17**),[5] strophathojavoside (**18**),[16] desglucocheirotoxin (**19**),[14] strophalloside (**20**),[13] convalloside (**21**),[17] glucostrophalloside (**22**),[18] malayoside (**23**),[5] antiarigenin (**24**),[13] α-antiarin (**25**),[5] antialloside (**26**),[16] toxicarioside B (**27**),[7] and β-antiarin (**28**)[13] were also isolated from *A. toxicaria* trunk bark. These known compounds were identified by comparison of their physical and spectroscopic properties with those reported in the literature.

Minimal positive effective concentrations ($PIEC_{min}$) to increase contraction of rat left atria were 0.05, 0.25, 1, and 7.5 μM for ouabain, **23**, **16**, and **8**, respectively. In right ventricular muscle, the $PIEC_{min}$ for the positive inotropic action of different cardiac glycosides varied from 0.075 to 8.33 μM (Table 3). Compounds with lower $PIEC_{min}$ may have stronger binding affinity to Na^+/K^+-ATPase of cardiac muscle.

Maximal contractions after treatment with cardiac glycosides are expressed as a percentage of those before glycoside treatment. For ouabain, **23**, **16**, and **8**, these values were 775%, 660%, 165%, and 144%, respectively, in left atria. In right ventricular muscle, the maximal contractions were 249%, 446%, 240%, and 260% compared to basal values for ouabain, **23**, **16**, and **8**, respectively (Table 4).

The safety index (therapeutic index) was calculated from the ratio of the arrhythmogenic concentration to the minimal effective positively inotropic concentration. A narrow margin of safety index restricts the therapeutic use of this class of positive inotropic drugs. For example, the safety index of digitalis is narrow, and arrhythmias are common problems in clinical practice.[19] Safety indexes were 20, 20, 9, and 7.5 for ouabain, **23**, **16**, and **8**, respectively, in left atria. Safety indexes of ouabain, **23**, **16**, and **8** were 20, 24, 8.7, and 9.7, respectively, in right ventricular muscle (Table 5).

Other compounds, such as **2** and **13**, had larger safety indexes than ouabain and **23** (100 and 65 for **2** and **13** versus 20 and 24 for ouabain and **23**). Maximal contractions after treatment with **2** and **13** were 382% and 408%, respectively. In our previous study, we found that **23** had a larger safety index than ouabain in vivo.[20]

Table 3. Minimal Positive Inotropic Effectctive Concentration ($PIEC_{min}$) of Cardiac Glycosides in Atria and Ventricular Strips

left atria strips		right ventricular strips	
cmpd	$PIEC_{min}$ (μM)	cmpd	$PIEC_{min}$ (μM)
1	0.10 ± 0.07	**1**	0.083 ± 0.03
2	0.38 ± 0.13	**2**	0.075 ± 0.05
3	2.75 ± 0.25	**3**	4 ± 1
4	2.75 ± 0.25	**4**	2.75 ± 0.25
5	0.42 ± 0.08	**5**	1.33 ± 0.6
6	1.42 ± 0.58	**6**	3.17 ± 0.93
7	3.75 ± 1.25	**7**	3.67 ± 1.33
8	7.50 ± 2.50	**8**	8.33 ± 1.67
9	0.83 ± 0.17	**9**	0.67 ± 0.17
10	0.1	**10**	
11	0.05 ± 0.01	**11**	0.25 ± 0.1
12	0.1	**12**	0.25
13	1.25 ± 0.75	**13**	0.30 ± 0.20
14	0.1	**14**	0.25
16	1	**16**	4.8 ± 1.5
17	0.1	**17**	0.125
18	0.25	**18**	1
19	0.05	**19**	0.5
20	0.5	**20**	0.5
21	0.25	**21**	1
22	0.25	**22**	1
23	0.25	**23**	0.25
24	0.05	**24**	
25	0.5	**25**	2
26	0.25	**26**	
27	0.1	**27**	
28	1	**28**	2
ouabain	0.05	ouabain	0.05

Table 4. Maximal Positive Inotropic Effect (PIE_{max}) of Cardiac Glycosides in Atria and Ventricular Strips

left atria strips		right ventricular strips	
cmpd	PIE_{max} (% of basal)	cmpd	PIE_{max} (% of basal)
1	158 ± 28	**1**	165 ± 33
2	373 ± 187	**2**	382 ± 99
3	208 ± 34	**3**	229 ± 35
4	201 ± 1	**4**	369 ± 47
5	264 ± 38	**5**	230 ± 19
6	140 ± 28	**6**	618 ± 321
7	419 ± 31	**7**	221 ± 69
8	144 ± 27	**8**	260 ± 33
9	562 ± 236	**9**	1246 ± 49
10	367	**10**	-
11	381 ± 104	**11**	355 ± 33
12	278	**12**	150
13	205 ± 5	**13**	408 ± 128
14	138	**14**	625
16	165 ± 15	**16**	240 ± 74
17	467	**17**	300
18	567	**18**	200
19	300	**19**	300
20	225	**20**	350
21	443 ± 105	**21**	292 ± 17
22	489	**22**	350
23	660 ± 88	**23**	464 ± 89
24	121	**24**	
25	220	**25**	200
26	350	**26**	
27	163	**27**	
28	243	**28**	250
ouabain	775 ± 128	ouabain	249 ± 26

Table 5. Safety Index of Cardiac Glycosides in Atria and Ventricular Strips

left atria strips			right ventricular strips		
cmpd	safety	index	cmpd	safety	index
1	4.3 ± 0.3		**1**	18 ± 11.1	
2	5.0 ± 1.0		**2**	100 ± 60	
3	15		**3**	9.7 ± 3.7	
4	7.3 ± 0.7		**4**	6.3 ± 0.3	
5	10		**5**	7.7 ± 3.5	
6	6.0 ± 2.3		**6**	5.8 ± 1.0	
7	13 ± 1		**7**	20.7 ± 10	
8	7.5 ± 2.5		**8**	9.7 ± 3.3	
9	6.7 ± 1.7		**9**	10.7 ± 1.8	
10	1000		**10**		
11	11 ± 3.7		**11**	8.8 ± 3.3	
12	10		**12**	2	
13	6.3 ± 3.8		**13**	65 ± 25	
14	5		**14**	20	
16	9		**16**	8.7 ± 3.0	
17	10		**17**	8	
18	10		**18**	5	
19	50		**19**	10	
20	2.5		**20**	4	
21	20		**21**	9	
22	10		**22**	10	
23	20		**23**	24	
24	20		**24**		
25	10		**25**	15	
26	80		**26**		
27	10		**27**		
28	10		**28**	15	
ouabain	20		ouabain	20	

Whether **2** and **13** have better safety indexes than ouabain or **23** in animals remains to be determined.

The following structure−activity relationships were identified in these studies. Changing the *β*-*O*-α-rhamnose in **7** to *β*-*O*-*β*-antiarose in **8** increased $PIEC_{min}$ in atria from 3.75 μM to 7.5 μM and in right ventricular muscle from 3.67 μM to 8.33 μM (Table 3). Comparison of **3** with one sugar (α-*O*-α-rhamnose) and **4** with two sugars [α-*O*-α-rhamnosyl(4→1)*β*-glucose] showed a decrease in $PIEC_{min}$ from 4 μM to 2.75 μM, in right ventricular muscles. Substitution of the C-18 CH_3 of **11** with CH_2OH in **14** increased $PIEC_{min}$ in atria from 0.05 μM to 0.1 μM, but $PIEC_{min}$ in right ventricular muscle remained at 0.25 μM (Table 3). Similarly, changing the C-18 CHO of **23** to COOH in **5** increased $PIEC_{min}$ from 0.25 μM to 0.42 μM in atria and from 0.25 μM to 1.33 μM in right ventricular muscle. Finally, glycosylation of the carboxylic acid increased $PIEC_{min}$ in atria from 0.42 μM (**5**, COOH) to 1.42 μM (**6**, COOglc).

To confirm that, like digitalis, **23** exerts an inotropic effect through inhibition of Na^+/K^+-ATPase, the sodium pump current (I_{pump}) was measured by the whole-cell patch clamp technique. I_{pump} currents before and after **23** treatment were recorded. Figure 1 shows basal I_{pump} (filled squares), I_{pump} with **23** at 1 μM (filled triangles), and I_{pump} with **23** at 3 μM (filled circles). Compound **23** inhibited the sodium pump current in a concentration-dependent manner. A detailed mechanistic study of the inotropic effect of **23** in guinea pig has been reported.[20]

Several noteworthy conclusions were obtained from this study. Nine new cardiac glycosides (**1**−**9**) and one aglycone (**10**), together with 18 known cardiac glycosides/aglycones, were isolated from *A. toxicaria*. The first compounds with COOH (**5**, **7**, **8**) and COOglc (**6**) from this plant were reported. These groups are representative units for the metabolic pathway of cardiac glycosides. A side-by-side evaluation of biological activity properties of the isolated cardiac glycosides provided additional insights into the pharmacological profile of this compound class. Our data showed that α-*O*-α-rhamnosyl(4→1)-*β*-glucose linked at the C-3 OH resulted in better $PIEC_{min}$ in right ventricular muscle. The presence of $-CH_3$ at C-10 was better than $-CH_2OH$, −CHO, and −COOH, as measured by $PIEC_{min}$, in atria and right ventricular muscle. Increasing the polarity of this substituent may be beneficial in cardiac glycosides. Most significantly, **23** increased contractility and inhibited sodium pump current in guinea pig heart preparations in a concentration-dependent manner, and the safety indexes of **2**, **13**, and **23** were better than those of ouabain in vitro.

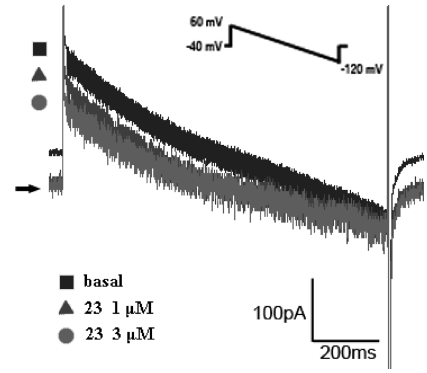

Figure 1. Drug effects on I_{pump} of basal (filled squares), **23** at 1 μM (filled triangles), and 3 μM (filled circles) treated cells.

Experimental Section

General Experimental Procedures. Proton NMR spectra were recorded on Bruker Avance 300 (300 MHz) and AMX 400 (400 MHz) spectrometers. The chemical shifts (ppm) were measured with tetramethylsilane (TMS) as internal standard and deuterated pyridine as solvent. Mass spectra were performed in the EI mode on a VG 70−250S spectrometer. The optical rotation was recorded on a JASCO DIP-370 polarimeter. Merck silica gel 60 (Merck 70-230, 230−400 mesh) was used for column chromatography. Glass sheets of silica gel 60 F_{254} (Merck 0.2 mm thick) were used for TLC. Melting points were measured on a Yanagimoto MP-S3 micromelting point apparatus and are uncorrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer, and IR spectra were determined as KBr discs on a Shimazu FTIR-8501 spectrophotometer.

Plant Material. Trunk bark of *A. toxicaria* was collected from Yunnan, China, and authenticated by C. S. Kuoh (Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan). A voucher specimen (NCKUWu 92012) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan, R.O.C.

Extraction and Isolation. The trunk bark of *A. toxicaria* (6.0 kg) was cut into small pieces and extracted with 95% EtOH (20 L × 3). Evaporation of the solvent under reduced pressure provided 239.0 g of crude extract, which was partitioned between $CHCl_3-H_2O$ and *n*-BuOH−H_2O, successively, to yield $CHCl_3$ (65.1 g), *n*-BuOH (100.2 g), and H_2O (73.7 g) fractions. The $CHCl_3$ fraction was subjected to silica gel CC using increasing polarity mixtures of *n*-hexane−acetone as eluant to give 14 fractions. Fraction 6 was chromatographed on silica gel using diisopropyl ether−MeOH (40:1) to obtain **10** (8.7 mg) and **16** (103.2 mg). Fraction 7 was chromatographed on silica gel and eluted with $CHCl_3$−MeOH (15:1) to give **13** (6.6 mg) and **18** (7.0 mg). Fraction 8 was chromatographed on silica gel using EtOAc−MeOH (20:1) to obtain **5** (37.6 mg), **15** (3.2 mg), and **27** (8.8 mg), successively. Fraction 9 was chromatographed on silica gel using EtOAc−MeOH (20:1) to afford **23** (21.4 mg). Fraction 10 was chromatographed on silica gel using EtOAc−MeOH (20:1) to yield **17** (117.2 mg).

The *n*-BuOH fraction was subjected to Diaion HP-20 CC eluting with a H_2O−MeOH gradient system to give 12 fractions. Fraction 5 was chromatographed on silica gel using $CHCl_3$−MeOH (9:1) to obtain **7** (34.7 mg), **8** (147.4 mg), **9** (1.5 mg), **24** (58.9 mg), **25** (11.0 mg), **26** (4.3 mg), and **28** (7.3 mg), successively. Fraction 6 was chromatographed on silica gel using $CHCl_3$−MeOH (9:1) to obtain **25** (118.4 mg). Fraction 7 was chromatographed on silica gel using $CHCl_3$−MeOH−H_2O (9:1:0.05) to obtain **21** (11.4 mg). Fraction 8 was chromatographed on silica gel using $CHCl_3$−MeOH−H_2O (9:1:0.05) to afford **2** (12.3 mg), **3** (112.3 mg), **4** (12.2 mg), **6** (5.9 mg), **12** (9.1 mg), **14** (6.1 mg), **17** (431.2 mg), **18** (4.3 mg), **20** (5.9 mg), **21** (79.8 mg), and **22** (18.3 mg). Fraction 9 was chromatographed on silica gel

using $CHCl_3$−MeOH−H_2O (9:1:0.05) to obtain **11** (7.6 mg), **17** (12.5 mg), **19** (19.7 mg), and **20** (14.3 mg). Fraction 10 was chromatographed on silica gel using $CHCl_3$−MeOH−H_2O (9:1:0.05) to obtain **1** (9.8 mg), **11** (3.3 mg), **19** (10.2 mg), **20** (3.5 mg), and **27** (13.5 mg).

The water fraction was directly subjected to Diaion HP-20 CC eluting with water containing increasing proportions of MeOH to give 12 fractions. Fraction 8 was chromatographed on a Sephadex LH-20 column using mixtures of MeOH−H_2O of increasing polarity to obtain **25** (20.2 mg) and **26** (1.3 mg). Fraction 9 was chromatographed on Sephadex LH-20 eluting with water containing increasing proportions of MeOH to give **4** (26.4 mg), **17** (43.2 mg), and **21** (26.8 mg).

Antiaroside A (1): colorless needles ($CHCl_3$−MeOH); mp 184−186 °C; $[\alpha]^{25}_D$ −24.4 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.67) nm; IR (KBr) ν_{max} 3450, 2939, 1738, 1622, 1450, 1383, 1078, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 559 $[M + 23]^+$ (16), 537 (47), 391 (29), 373 (43), 355 (100), 337 (54), 277 (47), 185 (98); HRFABMS *m/z* 537.3063 $[M + 1]^+$ (calcd for $C_{29}H_{45}O_9$, 537.3064).

Antiaroside B (2): colorless syrup; $[\alpha]^{25}_D$ −20.09 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.32) nm; IR (KBr) ν_{max} 3400, 2936, 1738, 1730, 1655, 1067, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 737 $[M + 39]^+$ (3), 721 $[M + 23]^+$ (3), 699 (2), 519 (3), 356 (5), 185 (100), 147 (25); HRFABMS *m/z* 737.8260 $[M + 39]^+$ (calcd for $C_{35}H_{54}KO_{14}$ 737.8262).

Antiaroside C (3): colorless powder ($CHCl_3$−MeOH); mp 231−232 °C; $[\alpha]^{25}_D$ −11.31 (*c* 1.03, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.17) nm; IR (KBr) ν_{max} 3440, 2934, 1734, 1715, 1618, 1454, 1344, 1198, 1057 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 551 $[M + 1]^+$ (25), 405 (13), 387 (35), 369 (36), 351 (18), 341 (22), 323 (26), 185 (100), 179 (12), 147 (54); HRFABMS *m/z* 551.2855 $[M + 1]^+$ (calcd for $C_{29}H_{43}O_{10}$, 551.2856).

Antiaroside D (4): colorless syrup; $[\alpha]^{25}_D$ −21.73 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.37) nm; IR (KBr) ν_{max} 3400, 2932, 1734, 1647, 1456, 1067, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 751 $[M + 39]^+$ (7), 549 (2), 403 (6), 387 (6), 369 (6), 359 (4), 342 (6), 341 (8), 323 (12), 207 (16), 185 (100), 179 (3), 163 (7), 147 (19); HRFABMS *m/z* 751.2943 $[M + 39]^+$ (calcd for $C_{35}H_{52}KO_{15}$, 751.2943).

Antiaroside E (5): colorless syrup; $[\alpha]^{25}_D$ −29.07 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.40) nm; IR (KBr) ν_{max} 3460, 2936, 1738, 1670, 1453, 1076, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 551 $[M + 1]^+$ (30), 507 (10), 462 (17), 417 (38), 387 (23), 359 (15), 341 (26), 323 (35), 315 (57), 277 (25), 185 (100), 147 (63); HRFABMS *m/z* 551.2856 $[M + 1]^+$ (calcd for $C_{29}H_{43}O_{10}$, 551.2856).

Antiaroside F (6): colorless syrup; $[\alpha]^{25}_D$ −19.61 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.42) nm; IR (KBr) ν_{max} 3400, 1726, 1655, 1647, 1642, 1545, 1533, 1460 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 751 $[M + 39]^+$ (7), 713 (3), 550 (3), 534 (2), 490 (3), 241 (6), 185 (100), 147 (6); HRFABMS *m/z* 751.2943 $[M + 39]^+$ (calcd for $C_{35}H_{53}O_{15}$, 751.2945).

Antiaroside G (7): colorless syrup; $[\alpha]^{25}_D$ +3.07 (*c* 0.3472, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.67) nm; IR (KBr) υ_{max} 3440, 2941, 1738, 1726, 1514, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 583 $[M + 1]^+$ (11), 437 (6), 383 (3), 277 (9), 241 (7), 207 (9), 185 (100), 149 (18), 147 (6), 115 (116); HRFABMS *m/z* 583.2752 $[M + 1]^+$ (calcd for $C_{29}H_{43}O_{12}$, 583.2754).

Antiaroside H (8): colorless syrup; $[\alpha]^{25}_D$ +0.77 (*c* 1.47, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.20) nm; IR (KBr) υ_{max} 3420, 2970, 2941, 2878, 1739, 1710, 1618, 1450, 1416, 1377, 1313, 1030, 993 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 583 $[M + 1]^+$ (21), 437 (25), 401 (8), 383 (10), 337 (11), 185 (100), 147 (28), 129 (33); HRFABMS *m/z* 583.2753 $[M + 1]^+$ (calcd for $C_{29}H_{43}O_{12}$, 583.2754).

Antiaroside I (9): colorless syrup; $[\alpha]^{25}_D$ −28.22 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.17) nm; IR (KBr) ν_{max} 3430, 2934, 1742, 1647, 1454, 1364, 1225, 1049, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 523 $[M + 1]^+$ (28), 360 (11), 359 (25), 341 (30), 323 (14), 225 (39), 185 (100), 147 (27), 131 (56), 129 (36); HRFABMS *m/z* 523.2908 $[M + 1]^+$ (calcd for $C_{28}H_{43}O_9$, 523.2907).

Antiarotoxinin A (10): colorless powder; $[\alpha]^{25}_D$ +30.96 (*c* 0.087, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.95) nm; IR (KBr) ν_{max} 3304, 2943, 1761, 1643, 1275, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 408 (M^+, 2), 390 (9), 372 (25), 354 (26), 318 (100), 219 (29), 201 (43), 145 (21), 124 (39), 121 (28), 111 (37), 109 (26), 107 (36), 93 (40), 91 (46), 81 (55), 55 (60); HREIMS *m/z* 408.2513 $[M]^+$ (calcd for $C_{23}H_{36}O_6$, 408.2511).

Assay Methods of Positive Inotropic Action and Arrhythmogenic Action. Adult guinea pigs (300−500 g) were anesthetized with pentobarbital (25 mg/kg, ip). The heart was excised, and retrograde coronary perfusion was performed with normal Tyrode's solution containing (in mM) NaCl 137, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1.1, NaH_2PO_4 0.33, $NaHCO_3$ 11.9, and glucose 11 through a coronary artery. Tyrode's solution was maintained at 37 °C and continuously aerated with 95% O_2 + 5% CO_2 (pH 7.2−7.4 under these conditions). Left atria and right ventricular muscles were separated from the heart. One end of the muscle was attached to a rigid support, and the other end was attached to a transducer in the 10 mL bath. Each tissue was placed under 1 g of tension and stimulated at 2 Hz, with pulses of 2 ms duration and amplitude twice the threshold. Following stabilization for about 60 min, drugs were cumulatively added. The positive inotropic effects (PIE_{max}) and safety indexes were studied according to the methods described previously.[20,21] Briefly, the minimal positive inotropic effective concentration ($PIEC_{min}$) to increase myocardial contraction and the arrhythmogenic concentration to induce arrhythmia in these isolated cardiac preparations were measured. The maximal positive inotropic effect was determined at a concentration level immediately before the occurrence of cardiac arrhythmia, and the safety index was then measured from the ratio of arrhythmogenic concentration to minimal effective positively inotropic concentration.

Electrophysiological Recording of Malayoside (23). Cardiomyocytes were isolated by using the enzymatic method previously described.[20] Adult male guinea pigs (200−250 g) were intraperitoneally injected with sodium pentobarbital (25 mg/kg) plus heparin (16 mg/kg). After the guinea pig was deeply anesthetized, the heart was excised and the coronary artery was antegradely perfused with oxygenated Ca^{2+}-free HEPES solution containing (in mM) NaCl 137, glucose 22, HEPES 6, $MgSO_4$ 1.2, KH_2PO_4 1.2, and KCl 5.4; pH was adjusted to 7.4 using NaOH. The heart was then perfused with the same solution containing 0.4 mg/mL collagenase (type II, Sigma Chemical Co., St. Louis, MO), 0.06 mg/mL protease (type XIV, Sigma), and bovine serum albumin (1 mg/mL).

After 4−5 min of digestion, enzymes were washed out in Kruftbruhe solution containing (in mM) taurine 10, oxylate 10, glutamate 70, KCl 25, KH_2PO_4 10, glucose 11, EGTA 0.5; pH was adjusted to 7.4 using KOH. The ventricles were then chopped, resuspended under gentle mechanical agitation, and stored in Kruftbruhe solution at room temperature.

The whole-cell patch clamp technique was used to record ionic currents in voltage clamp mode with a Dagan 8900 voltage clamp amplifier (Dagan Co., Minneapolis, MN). A droplet of the cell suspension was placed in a chamber mounted on the stage of an inverted microscope (Nikon, Diaphot, Japan). After settling, cells were finally exposed to the bath solution containing (in mM) NaCl 137, KCl 5.4, $MgCl_2$ 2.9, HEPES 6, glucose 22, NaH_2PO_4 0.33, $BaCl_2$ 2, and CdCl 0.2; pH was adjusted to 7.4 using NaOH. For the measurement of I_{pump}, a pipet was filled with the internal solution containing (mM) CsOH 80, NaOH 50, $MgCl_2$ 3, TEA-Cl 20, aspartic acid 100, HEPES 10, ATP-Mg 10, GTP-Na_3 0.2, glucose 5.5, Na-creatine phosphate 5, and pyruvic acid 5; pH was adjusted to 7.2 using CsOH. Heat-polished glass electrodes (tip resistances about 1 MΩ when filled with pipet internal solution) were used. After rupture of the patch, the holding potential was set at −40 mV to inactivate Na^+ channels and the cell interior was allowed to equilibrate 5 min with the pipet solution. Then membrane currents were elicited by voltage ramps from +60 mV to −120 mV. Junction potentials were zeroed before the formation of the membrane−pipet seal in the bath solution. The series resistance was electronically compensated by about 80% to minimize the duration of the capacitive surge on the current recorded and the voltage drop across the pipet. Currents were elicited and acquired using a Digidata 1200 data acquisition system controlled using pClamp software (Axon Instruments). Recordings were lowpass filtered at 10 kHz and stored on the hard disk of a computer.

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Supporting Information Available: NMR spectra of new compounds **1**−**10** and structures of known compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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