Three New Cardenolides from Methanol Extract of Stems and Twigs of *Nerium oleander*

Liming BAI,*[,a] Ming ZHAO,[a] Asami TOKI,[b] Jun-ichi SAKAI,[c] Xiao-yang YANG,[d] Yuhua BAI,[e] Mariko ANDO,[f] Katsutoshi HIROSE,[g] and Masayoshi ANDO*[,c,1)]

[a] *College of Chemistry and Chemistry Engineering, Qiqihar University; 30 Wenhuadajie, Qiqihar, Heilongjian Province 161006, China:* [b] *Graduate School of Science and Technology, Niigata University;* [c] *Department of Chemistry and Chemical Engineering, Faculty of Engineering, Niigata University; 2–8050 Ikarashi, Nishi-ku, Niigata 950–2181, Japan:* [d] *Atmospheric Chemistry & Aerosol Division, Chinese Research Academy of Environmental Science; No. 8 Anwai Beiyuan Dayangfang, Chaoyang District, Beijing 100012, China:* [e] *Department of Medicinal Chemistry, Pharmaceutical Department, Daqing Campus of Harbin Medical University; Daqing 163319, China:* [f] *Technical Division, School of Engineering, Tohoku University; 6–6 Aramaki-aza-Aoba, Aoba-ku, Sendai 980–8579, Japan: and* [g] *KNC Laboratories Co., Ltd.; 3–2–34 Takatukadai, Nishi-ku, Kobe, Hyogo 651–2271, Japan.*

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Two new cardenolide monoglycosides, cardenolides B-1 (1) and B-2 (2) were isolated from *Nerium oleander*, together with oleagenin (3) which is the first isolated compound from natural sources. The structure of compounds 1—3 were established on the basis of their spectroscopic data.

Key words *Nerium oleander*; cardenolide B-1; cardenolide B-2; oleagenin

Nerium oleander L. is a medium-sized evergreen flowering tree of 2—5 m in height and is planted throughout Japan as a garden and roadside tree. This species was distributed originally in the Mediterranean region, sub-tropical Asia, and the Indo–Pakistan subcontinent. Cardenolides in the leaves,[2—9)] roots, and root bark[10—13)] of this plant were investigated because of the interests in their biological activities.[14)] The cardiac glycoside digitoxin and digoxin have been used in treatment of cardiac diseases for many years,[14),15)] but they have a narrow therapeutic window because of arrhythmia and disturbance of atrio-ventricular contraction. Anticancer utilization of digitoixin, digoxin, and related cardenolides has been also investigated.[16,17)] We recently reinvestigated the cardenolide monoglycosides from *N. oleander* and isolated thirteen kinds of compounds, four of which were new compounds.[18)] Further chemical investigation on *N. oleander* has led to the isolation of two new cardenolides monoglycoside named cardenolides B-1 (**1**) and B-2 (**2**), and oleagenin (**3**) which is the first isolated compound from natural sources.

Results and Discussion

A methanol extract of air-dried stems and twigs of *N. oleander* was partitioned successively with hexane, ethyl acetate (EtOAc), and *n*-BuOH. From the less polar fraction of the extract with EtOAc, we have already reported three cardenolide sarmentosides and eight cardenolid diginosides including four new compounds, the major component of which was odoroside A (**17**) (0.018%).[18)] In this time, we isolated two new cardenolide monoglycosides, cardenolides B-1 (**1**) and B-2 (**2**), and oleagenin (**3**) from more polar fraction of the extract with EtOAc using silica gel column chromatography and reversed-phase HPLC.

Cardenolide B-1 (**1**) gave the elemental composition, $C_{30}H_{44}O_8$, which was determined by high resolution (HR)-FAB-MS analysis. The IR spectrum of **1** indicated the presence of hydroxyl (3539 cm^{-1}) and α,β-unsaturate-γ-lactone (1786, 1751, 1631 cm^{-1}) groups. The ^{13}C-NMR spectrum displayed 30 carbon signals (Table 1). A carbonyl carbon resonated at δ 173.6 and two olefin carbon resonances were located at δ 169.5 (qC) and 116.9 (CH). Four resonances for carbons bearing oxygen were observed at δ 73.2 (CH_2), 73.7 (CH), 70.5 (qC), and 65.3 (qC) in addition to one methoxy methyl and five oxygenated carbon signals of a 6-deoxyhexose sugar. From the distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectra, the remaining carbon resonances were three methyl, nine methylene, three methine, and two quaternary carbons. The ^{1}H-NMR spectra showed two methyl singlets (δ 0.85, 1.01) and one additional methyl doublet from the sugar portion at 1.36 (d, J=6.3 Hz). The connectivity of the protonated carbons (C-1 through C-7; C-9, C-11, and C-12; C-15 through C-17) was determined from the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum. A heteronuclear multiple bond connectivity (HMBC) experiment was used to determine the carbon–carbon connection through the nonprotonated carbon atoms [HMBC correlations: H-17 (δ 2.57) to C-12, C-13 (δ 41.8, qC), C-15, C-18, C-20 (δ 169.5, qC), C-21 (δ 73.2, CH_2), and C-22 (δ 116.9, CH); CH_3-18 (δ 0.85) to C-12, C-13 (δ 41.8, qC), C-14 (δ 70.5, qC), and C-17; CH_3-19 (δ 1.01) to C-1, C-5, C-9, and C-10 (δ 36.7, qC); H-11α and β (δ 1.15, 1.26) to C-8 (δ 65.3, qC), C-9, and C-12]. Interpretation of these results suggests that compound **1** has steroid A, B, C, and D rings[19,20)] bearing an 8,14-epoxide ring, and an α,β-unsaturated γ-lactone moiety at C-17. The HMBC correlations [H-3 to C-2, C-5, and C-1′; H-1′ to C-3] were used to place an *O*-glycosyl bond at C-3. The chemical shift values of C-8 (δ 65.3) and C-14 (δ 70.5) of **1** are in good accordance with those of analogous epoxides **18**[18)] [C-8 (δ 65.2) and C-14 (δ 70.1)] and **19**[5,18,21)] [C-8 (δ 65.3) and C-14 (δ 70.5)] but different from those of diol **15**[5,18)] [C-8 (δ 77.2) and C-14 (δ 85.9)]. The sugar portion of **1** was assigned to digitalose on the basis of comparisons of the ^{13}C- and ^{1}H-NMR data of **1** (Table 1) with those of an analogous compound such as **4**[22)] [^{13}C-NMR: δ 73.9 (C-3), 101.1 (C-1′), 70.8 (C-2′), 82.8 (C-3′), 68.2 (C-4′), 70.3 (C-5′), 16.4 (C-6′), 57.5 (OMe); ^{1}H-NMR:

* To whom correspondence should be addressed. e-mail: baiyun68066677@yahoo.co.jp; andomasa@kdp.biglob.ne.jp

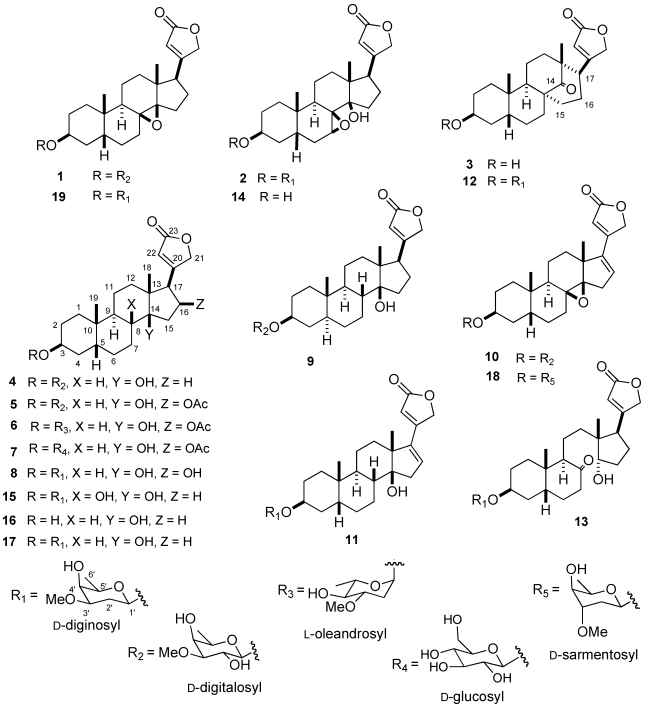

Fig. 1. Structure of Compounds **1**—**19**

δ 4.04 (H-3, br s, $W_{h/2}$=7.5 Hz), 4.24 (H-1′, d, J=7.6 Hz), 3.65 (H-2′, dd, J=10.3, 7.6 Hz), 3.21 (H-3′, dd, J=7.6, 3.3 Hz), 3.84 (H-4′, br d, J=3.3 Hz), 3.56 (H-5′, br q, J=6.6 Hz), 1.34 (H-6′, d, J=6.6 Hz), 3.52 (OCH_3, s)]. Since only D-digitalose is known in *N. oleander*, the sugar in **1** is regarded as D-digitalose. The conclusion is supported by the coupling constants of ^{1}H-NMR spectrum (Table 1) and the nuclear Overhauser effect spectroscopy (NOESY) correlations (H-1′ to H-3′ and H-5′; H-3′ to H-1′, H-4′, and H-5′; H-4′ to H-3′ and H-5′; H-5′ to H-1′, H-3′, and H-4′; H-6′ to H-4′ and H-5′) of sugar moiety of **1**. NOESY correlations [CH_3-19 with H-5; H-4α with H-7α and H-9] suggested AB-*cis* ring junction in **1**. The β-configuration of the 8,14-epoxide ring of **1** was strongly suggested by the fact that the chemical shits of C-8 and C-14 are in good accordance with those of known cardenolides with 8β,14β-epoxide ring as mentioned above. This was also supported by NOE correlation of H-15α with H-7α and H-7β. In ^{1}H-NMR-spectra, the small coupling constant of H-3 ($W_{h/2}$=7.5 Hz) was in good agreement with that of α(eq)-H at C-3 of 5β steroids. The above-mentioned spectroscopic analyses and NOESY correlations [CH_3-19 with H-6β, and 11β; H-11β with H-12β and CH_3-18; CH_3-18 with H-21a and H-22; H-12α with H-9 and H-17; H-17 with H-15α and H-16α; H-16β with H-22 and CH_3-18] indicated the relative stereochemistry of **1** to be 3β-*O*-(β-D-digitalosyl)-8,14-epoxy-5β,14β-card-20(22)-enolide. Since all known cardenolides isolated from *N. oleander* possess the same absolute configuration in genin moiety as shown in structures **4**, **15**, **18** and **19**, the absolute configuration of **1** is regarded as (3*S*, 5*R*, 8*S*, 9*R*, 10*S*, 13*R*, 14*R*, 17*R*). This conclusion was also supported by the fact that $[\alpha]_D$ sign of **1**{$[\alpha]_D^{20}$ +28.57 (c=0.392, $CHCl_3$)} is the same as those of 3β-*O*-(β-D-digitalosyl)- and 3β-*O*-(β-D-diginosyl)-cardenolides with analogous structures such as **4** {$[\alpha]_D^{20}$ +5.57 (c=0.56, MeOH)} and **19** {$[\alpha]_D^{20}$ +13.4 (c=0.55, $CHCl_3$)}.

Cardenolides B-2 (**2**) had the composition, $C_{30}H_{44}O_8$, which was determined by HR-FAB-MS analysis. Similar IR data were obtained for compound **2** as compared to compound **1**. The ^{13}C-NMR spectrum displayed 30 carbon signals (Table 1). The 3β-*O*-(glycosyl)-5β,14β-card-20(22)-enolide structure of **2** was confirmed by analogous NMR analysis of **2** with that of **1**. ^{1}H- and ^{13}C-NMR spectra of the sugar portion of **2** are different from those of **1**. The coupling constants [H-1′ ($J_{1',2'\beta}$=9.8 Hz and $J_{1',2'\alpha}$=1.7 Hz), H-3′ ($J_{3',2'\beta}$=12.1 Hz, $J_{3',2'\alpha}$=4.8 Hz, and $J_{3',4'}$=3.2 Hz), and CH_3-6′ (d J=6.6 Hz)] and NOESY correlations (H-1′ with H-2′α, H-3′ and H-5′; H-4′ with H-3′, H-5′, 6′ and 3′-OMe) of a 2,6-dideoxyhexose sugar of **2** suggested it to be diginose. The ^{13}C- and ^{1}H-NMR data of the sugar moiety of **2** were actually superimposable with 3β-*O*-(β-diginosyl)-moiety of **15**[5,18] [^{13}C-NMR: δ 97.7 (C-1′), 32.0 (C-2′), 78.0 (C-3′), 67.2 (C-4′), 70.4 (C-5′), 16.8 (C-6′), 55.7 (OMe); ^{1}H-NMR: δ 4.45 (H-1′, dd, J=9.8, 1.7 Hz), 1.93 (H-2′α, m), 1.71 (H-2′β, m), 3.34 (H-3′, ddd, J=12.0, 4.9, 3.2 Hz), 3.69 (H-4′, br s), 3.43 (H-5′, q, J=6.6 Hz), 1.32 (H-6′, d, J=6.6 Hz), 3.40 (OMe, s)] and **19**[5,18,21] [^{13}C-NMR: δ 97.7 (C-1′), 32.1 (C-2′), 78.0 (C-3′), 67. 1 (C-4′), 70. 3 (C-5′), 16. 9 (C-6′), 55.7 (OMe); ^{1}H-NMR: δ 4.47 (H-1′, dd, J=9.8, 2.0 Hz), 1.93 (H-2′α, m), 1.70 (H-2′β, m), 3.34 (H-3′, ddd, J=12.0, 4.9, 3.2 Hz), 3.69 (H-4′, br s), 3.43 (H-5′, q, J=6.6 Hz), 1.34 (H-6′, d, J=6.6 Hz), 3.40 (OMe, s)]. Since only D-diginose is known in *N. oleander*, the sugar in **2** is regarded as D-diginose. The A—B *cis* ring junction of **2** was confirmed by NOE correlations [CH_3-19 with H-1β, H-5, H-6β, and 11β; H-2α with H-9]. Existence of an epoxide ring between C-7 and C-8, and a hydroxyl group at C-14 were suggested by their chemical shift values and HMBC correlation [H-7 (δ 3.21) with C-6, C-8 (63.9, qC), C-14 (81.0, qC); H-6β (δ 2.30) with C-4, C-5, C-7 (δ 51.2, CH) and C8 (63.9, qC)]. The observed coupling constants of H-7 [H-7 with H-6α (J=5.9 Hz) and H-7 with H-6β (J=0 Hz)] are in good accordance with those deduced from the dihedral angles of H-7 with H-6α and H-6β. The β-olientation of the 7,8-epoxide ring was also supported by NOE correlation (H-7 with H-15β, H-6α, and the proton of 14-OH). The observed NOE correlation of the proton of 14-OH [δ 2.37 (1H, s, $W_{h/2}$=5.0 Hz)] with CH_3-18 and H-7 indicated that the hydroxyl group at C-14 is fixed by intramolecular hydrogen bond with the oxygen of eopxide ring between C-7 and C-8. Thus, 7,8-epoxide ring, 14-hydroxyl group, and CH_3-18 are located in *cis*-β-olientation. The analysis of NOESY correlations [H-3 with H-1′; H-9 with H-2α, H-4α, H-11α, and H-12α; H-12α with H-17; 14-OH with CH_3-18 and H-7; CH_3-18 with 12β, H-21, and H_2-22] indicated the full stereochemistry of **2** to be the 3β-*O*-(β-D-diginosyl)-7β,8-epoxy-14-hydroxy-5β,14β-card-20(22)-enolide. The absolute configuration of **2** is regarded (3*S*, 5*S*, 7*S*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*, 17*R*) by the same reason mentioned in the structure of **1**. This conclusion was also supported by the fact that observed and caluculated $[\alpha]_D$ values of **2** showed same negative sign.[23]

Compound **3** gave the elemental composition, $C_{23}H_{32}O_4$, which was determined by HR-FAB-MS analysis. The IR spectrum of **3** indicated the presence of hydroxyl (3399 cm^{-1}), α,β-unsaturate-γ-lactone (1748 cm^{-1}), and ketone (1692 cm^{-1}) groups. The ^{13}C-NMR spectrum displayed 23 carbon signals (Table 1). A carbonyl carbon resonated at δ

Table 1. ^{13}C- and ^{1}H-NMR Data of **1**—**3** (125 MHz for ^{13}C-NMR and 500 MHz for ^{1}H-NMR, δ in ppm J in Hz)[a)]

Position	**1** (in $CDCl_3$)		**2** (in $CDCl_3$)		**3** (in C_5D_5N)	
	δ_C, mult.	δ_H (mult., J)	δ_C, mult.	δ_H (mult., J)	δ_C, mult.	δ_H (mult., J)
1	30.4, CH_2	1.45 (1H, m) 1.49 (1H, m)	31.1, CH_2	1.43 (1H, m) 1.09 (1H, m)	31.6, CH_2	1.79 (1H, m) 1.58 (1H, m)
2	26.6, CH_2	α: 1.47 (1H, m) β: 1.82 (1H, m)	27.1, CH_2	α: 1.58 (1H, m) β: 1.80 (1H, m)	28.9, CH_2	α: 1.62 (1H, m) β: 1.70 (1H, m)
3	73.7, CH	4.07 (1H, br s, $W_{h/2}$=7.5)	71.9, CH	4.01 (1H, br s, $W_{h/2}$=7.5)	65.8, CH	4.32 (1H, br s, $W_{h/2}$=8.0)
4	30.0, CH_2	α: 1.80 (1H, m) β: 1.60 (1H, m)	32.7, CH_2	α: 1.35 (1H, m) β: 1.48 (1H, m)	34.5, CH_2	α: 1.85 (1H, m) β: 1.52 (1H, br dd, 14.2, 3.2)
5	36.6, CH	1.79 (1H, m)	33.6, CH	1.62 (1H, m)	37.1, CH	2.08 (1H, br d, 13.2)
6	24.5, CH_2	α: 1.30 (1H, m) β: 2.15 (1H, m)	27.9, CH_2	α: 1.47 (1H, m) β: 2.30 (1H, m)	24.8, CH_2	α: 1.12 (1H, m) β: 2.35 (1H, m)
7	26.7, CH_2	α: 1.78 (1H, m) β: 1.14 (1H, m)	51.2, CH	3.21 (1H, d, 5.9)	29.5, CH_2	α: 1.06 (1H, ddd, 13.9, 13.9, 4.6) β: 1.98 (1H, m)
8	65.3, qC		63.9, qC		49.1, qC	
9	36.7, CH	1.90 (1H, dd, 11.0, 4.6)	31.6, CH	2.23 (1H, m)	46.0, CH	2.51 (1H, br d, 8.3)
10	36.7, qC		33.6, qC		37.9, qC	
11	16.1, CH_2	α: 1.15 (1H, m) β: 1.26 (1H, m)	20.3, CH_2	α: 1.41 (1H, m) β: 1.56 (1H, m)	21.4, CH_2	α: 2.32 (1H, m) β: 1.72 (1H, m)
12	37.0, CH_2	α: 1.16 (1H, m) β: 1.58 (1H, m)	41.0, CH_2	α: 1.54(1H, m) β: 1.75 (1H, m)	42.7, CH_2	1.96 (2H, m)
13	41.8, qC		52.2, qC		47.5, qC	
14	70.5, qC		81.0, qC	2.37 (14-OH)	221.3, qC	
15	25.7, CH_2	α: 2.00 (1H, m) β: 1.74 (1H, m)	34.4, CH_2	α: 2.24 (1H, m) β: 1.77 (1H, m)	44.1, CH_2	α: 1.88 (1H, dd, 14.4, 6.1) β: 1.68 (1H, ddd, 14.4, 14.4, 6.8)
16	27.0, CH_2	α: 1.88 (1H, m) β: 1.98 (1H, m)	28.4, CH_2	α: 2.26 (1H, m) β: 1.96 (1H, m)	26.9, CH_2	α: 2.68 (1H, dddd, 15.1, 14.4, 7.1, 6.8) β: 1.38 (1H, br dd, 15.1, 6.8)
17	51.5, CH	2.57 (1H, dd, 11.2, 6.6)	50.6, CH	2.81 (1H, dd, 8.3, 5.7)	53.0, CH	2.97 (1H, br d, 7.1)
18	16.1, CH_3	0.85 (3H, s)	17.1, CH_3	0.90 (3H, s)	23.4, CH_3	0.91 (3H, s)
19	24.7, CH_3	1.01 (3H, s)	24.0, CH_3	0.95 (3H, s)	26.6, CH_3	0.81 (3H, s)
20	169.5, qC		173.6, qC		171.9, qC	
21	73.2, CH_2	α: 4.71 (1H, dd, 17.4, 1.0) β: 4.81 (1H, dd, 17.5, 1.7)	73.3, CH_2	α: 4.79 (1H, dd, 18.1, 1.2) β: 4.94 (1H, dd, 18.1, 1.2)	73.4, CH_2	α: 4.80 (1H, dd, 17.6, 1.7) β: 4.72 (1H, dd, 17.6, 1.7)
22	116.9, CH	5.88 (1H, br s)	117.8, CH	5.88 (1H, br s)	116.4 (d)	5.89 (1H, br s)
23	173.6, qC		174.2, qC		173.8, qC	
1′	101.3, CH	4.27 (1H, d, 7.8)	97.9, CH	4.43 (1H, dd, 9.8, 1.7)		
2′	70.8, CH	3.66 (1H, dd, 9.5, 7.8)	32.0, CH_2	α: 1.94 (1H, m) β: 1.69 (1H, m)		
3′	82.8, CH	3.22 (1H, dd, 9.5, 3.4)	78.0, CH	3.34 (1H, ddd, 12.1, 4.8, 3.2)		
4′	68.2, CH	3.85 (1H, br s)	67.2, CH	3.70 (1H, br s)		
5′	70.4, CH	3.57 (1H, br q, 6.3)	70.4, CH	3.42 (1H, q, 6.6)		
6′	16.2, CH_3	1.36 (3H, d, 6.3)	16.8, CH_3	1.32 (3H, d, 6.6)		
OMe	57.6, CH_3	3.53 (3H, s)	55.7, CH_3	3.40 (3H, s)		

a) Assighnment are based on DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC spectra.

221.3 and 173.8. Two olefin carbon resonances were located at δ 171.9 (qC) and 116.4 (CH). Two resonances for carbons bearing oxygen were observed at δ 73.4 (CH_2) and 65.8 (CH). From the DEPT and HMQC spectra, the remaining carbon resonances were two methyl, nine methylene, three methine, and three quaternary carbons. The ^{1}H-NMR spectra showed two methyl singlets (δ 0.91, 0.81). The connectivity of the protonated carbons (C-1 through C-7; C-9, C-11, and C-12; C-15 through C-17) was determined from the ^{1}H–^{1}H COSY spectrum. An HMBC experiment was used to determine the carbon–carbon connection through the nonprotonated carbon atoms [HMBC correlations: H-17 (δ 2.97) to C-12, C-13 (δ 47.5, qC), C-14 (δ 221.3, qC), C-16, C-18, C-20 (δ 171.9, qC), C-21 (δ 73.4, CH_2), and C-22 (δ 116.4, CH); CH_3-18 (δ 0.91) to C-12, C-13 (δ 47.5, qC), and C-14 (δ 221.3, qC); CH_3-19 (δ 0.81) to C-1, C-5, C-9, and C-10 (δ 37.9, qC); H-11α and β (δ 2.32, 1.72) to C-9, and C-12; H-15α and β (δ 1.88, 1.68) to C-7 and C-9]. Interpretation of these results suggested that compound **3** was a rearranged cardenolide with a 14-oxo-15(14→8)*abeo*-card-20(22)-enolide skeleton. NOESY correlations [CH_3-19 with H-5, H-6, and H-12β; H-2α with H-9α; H-4α with H-7α; H-9 with H-15α; H-11α with H-16α; CH_3-18 with H-12β and H-22; H-22 with H-15β] indicated full stereochemistry of **3** as (8*R*)-3β-hydroxy-14-oxo-15(14→8)*abeo*-5β-card-20(22)-enolide. Although the structure **3** is identical with that of aglycone of oleaside A that was obtained by acid hydrolysis,[3)] this is the first isolation of **3** from natural sources.

We isolated a further related ten cardenolides monoglycosides: odoroside H [3β-*O*-(β-D-digitalosyl)-14-hydroxy-5β,14β-card-20(22)-enolide] (**4**),[22)] neritaloside [3β-*O*-(β-D-digitalosyl)-16β-acetoxy-14-hydroxy-5β,14β-card-20(22)-enolide] (**5**),[22,26)] oleandrin [3β-*O*-(α-L-oleandrosyl)-16β-aceoxy-14-hydroxy-5β,14β-card-20(22)-enolide] (**6**),[5,6)] 3β-*O*-(β-D-glucosyl)-16β-acetoxy-14-hydroxy-5β,14β-card-20(22)-enolide (**7**),[27,28)] 3β-*O*-(β-D-diginosyl)-14,16β-dihydroxy-5β,14β-card-20(22)-enolide] (**8**),[12,29)] 3β-*O*-(β-D-digitalosyl)-14-hydroxy-5α,14β-card-20(22)-enolide (**9**),[10,12)] 3β-*O*-(β-D-dig-

italosyl)-8,14-epoxy-5β,14β-card-16,20(22)-enolide (**10**),[12,30] 3β-*O*-(β-D-diginosyl)-14-hydroxy-5β,14β-card-16,20(22)-enolide (**11**),[29] oleaside A [(8*R*)-3β-*O*-(β-D-diginosyl)-14-oxo-15(14→8)*abeo*-5β-card-20(22)-enolide] (**12**),[3] neriaside [3β-*O*-(β-D-diginosyl)-8,14-seco-14α-hydroxy-8-oxo-5β-card-20(22)-enolide] (**13**).[5,6] The most abundant component of this fraction is odoroside H (**4**) (0.008%).

Experimental

Melting points are uncorrected. Optical rotation values were measured using a Horiba Sepa-200 polarimeter. IR spectra were recorded on a Shimadzu FTIR-4200 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a Varian Unity-plus instrument at 500 and 125 MHz. ^{1}H-NMR assignments were determined by ^{1}H–^{1}H COSY experiments. ^{13}C-NMR assignments were determined using DEPT, HMQC, and HMBC experiments. HR-FAB-MS were recorded on a JEOL JMS-HX110 instrument. Silica gel (70—230 mesh) was employed for column chromatography and silica gel (230—400 mesh) for flash column chromatography. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10×250 mm stainless steel column and an Inertsil Prep-octadecyl functionalized silica gel (ODS) GL 10×250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

Plant Material The stems and twigs of *N. oleander* were collected in Niigata City, Niigata Province, Japan, in November 2001. The plant was identified by Dr. K. Yonekura, Department of Biology, Faculty of Science, Tohoku University, Sendai, Japan. A voucher specimen (2001-11-10) was deposited at the Department of Chemistry and Chemical Engineering, Niigata University.

Extraction and Isolation of Compounds 1—13 The air-dried stems and twigs (19.5 kg) were combined and extracted with MeOH (85 l) for 20 d. The MeOH extract was concentrated to 4 l and extracted with hexane (8×1000 ml). Water (1.3 l) was added to the MeOH layer, extracted with EtOAc (3×3000 ml), dried (Na_2SO_4), and concentrated to give an oily material (96.5 g). The water layer was further extracted with *n*-BuOH (3×500 ml), dried (Na_2SO_4), and concentrated to give an oily residue (53.76 g).

The EtOAc extract (96.5 g) was separated by column chromatography [silica gel (1.1 kg), a gradient of hexane, EtOAc, and MeOH] into five fractions, A—E. Fraction B [hexane–EtOAc (1 : 1), EtOAc], fraction C (EtOAc), and fraction D [EtOAc–MeOH (1 : 1)] gave on drying viscous oils, weighing 29.58 g, 23.33 g, and 32.15 g, respectively. The fraction B was dissolved in EtOAc (200 ml), stirred for 1 h, filtered, and concentrated to give viscous oil (19.86 g), which was further separated by column chromatography [silica gel (1 kg), a gradient of hexane, EtOAc, and MeOH] into 9 fractions, B1—B9. Fractions B7 [EtOAc (100%)], and B8 [EtOAc (100%)] gave on drying viscous oils [B7 (1.76 g), B8 (0.84 g)]. Fraction B7 was subjected to column chromatography [silica gel (300 g), gradient of hexane, EtOAc, and MeOH] to give five fractions, B71—B75. B73 (1.31 g) afforded compound **6** [53.51 mg (0.00027%)] by separation using HPLC [ODS, MeOH–MeCN–H_2O (1 : 1 : 2)]. B8 was subjected to column chromatography [silica gel (80 g), gradient of hexane, EtOAc, and MeOH] to give five fractions, B81—B85. B83 (296.0 mg) afforded compound **8** [9.7 mg (0.00005%)] by separation using HPLC [ODS, MeOH–MeCN–H_2O (1 : 3 : 5)]. Fraction C was subjected to flash column chromatography [silica gel (1 kg), hexane–EtOAc (1 : 59)] to give six fractions, C1—C6. Fraction C3 (8.65 g) was further separated by flash column chromatography [silica gel (800 g), hexane–EtOAc (3 : 7)] into four fractions, C31—C34. Fraction C33 (3.8 g) afforded compounds **3** [10.2 mg (0.000052%)] and **12** [132.3 mg (0.00068%)] by successive separation using HPLC [ODS, MeOH–MeCN–H_2O (1 : 6 : 9)], [ODS, MeOH–MeCN–H_2O (4 : 4 : 9)], and [ODS, MeOH–MeCN–H_2O (3 : 4 : 10)]. Fraction C34 (1.134 g) was divided into $CHCl_3$-soluble (C341) and $CHCl_3$-insoluble (C342) fractions. C342 (0.80 g) afforded compound **2** [13.9 mg (0.000071%)] by separation using HPLC [ODS, MeOH–MeCN–H_2O (4 : 4 : 10)]. Fraction C4 (0.96 g) was separated by flash column chromatography [silica gel (100 g), hexane–EtOAc (2 : 8)] into eight fractions, C41—C48. C47 was compound **4** [81.7 mg (0.00042%)]. Compound **11** [93.7 mg (0.00048%)] was obtained by crystallization of C43 from EtOAc. Fraction C5 (9.06 g) was separated by flash column chromatography [silica gel (900 g), hexane–EtOAc (1 : 10)] into three fractions, C51—C53. C51 was compound **4** [575.1 mg (0.00295%)]. Additional compound **4** [165.7 mg (0.00085%)] was obtained from C52 by crystallization from MeOH. Fraction C53 (2.16 g) was separated by HPLC [ODS, MeOH–MeCN–H_2O (4 : 4 : 10)] to give compounds **4** [704.0 mg (0.00362%)] and **5** [451.5 mg (0.00232%)]. Fraction C6 (851 mg) was separated by flash column chromatography [silica gel (90 g), EtOAc] into four fractions, C61—C64. Fraction C62 was crystallized from EtOAc to give compound **9** [107.2 mg (0.00055%)]. Fraction D was dissolved in EtOAc (200 ml), stirred for 1 h, filtered, and concentrated to give viscous oil (17.059 g), which was separated by column chromatography [silica gel (620 g), gradient of $CHCl_3$ and MeOH] into 12 fractions, D1—D12. Fraction D4 [$CHCl_3$–MeOH (98 : 2), 1.56 g] was further separated by flash column chromatography [silica gel (160 g), EtOAc] into six fractions, D41—D46. D42 (178 mg) was separated by silica gel HPLC [silica gel (20 g), EtOAc], followed by HPLC [ODS, MeOH–H_2O (55 : 45)] to give compound **13** [18.6 mg (0.00095%)]. The soluble portion of D43 (0.385 g) in EtOAc (D431, 0.314 g) was separated by HPLC [ODS, MeOH–H_2O (55 : 45)] to give compounds **4** [40.2 mg (0.00021%)], **5** [56.2 mg (0.00029%)], and **10** [46.7 mg (0.00024%)]. The insoluble portion of D43 in EtOAc (D432, 68 mg) was subjected to HPLC [ODS, MeOH–H_2O (55 : 45)] to give D4323 [**1**, 4.2 mg (0.00002%)], D4324, and D4325 [**10**, 9.6 mg (0.000049%)]. Separation of D4324 by HPLC [ODS, MeOH–MeCN–H_2O (1 : 1 : 2.5)] gave compounds **1** [5.4 mg (0.000028%)] and **10** [6.8 mg (0.000035%)].

Compound **7** (17.4 mg, 0.000089%) was obtained from the *n*-BuOH extract (53.76 g) by separation using column chromatography [silica gel, a gradient of $CHCl_3$ and MeOH], followed by HPLC [ODS, MeOH–MeCN–H_2O (1 : 2 : 7)].

3β-*O*-(β-D-Digitalosyl)-8,14-epoxy-5β,14β-card-20(22)-enolide (1) Colorless microcrystals, mp 203—206 °C (acetone–hexane); $[\alpha]_D^{20}$ +28.57 (c=0.392, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3539, 2936, 1786, 1751, 1631; UV (MeOH) nm (log ε): 222 (4.05); ^{1}H- and ^{13}C-NMR data are shown in Table 1; HR-FAB-MS m/z 533.3104 $[M+H]^+$ (Calcd for $C_{30}H_{45}O_8$, 533.3115).

3β-*O*-(β-D-Diginosyl)-7β,8-epoxy-14-hydroxy-5β,14β-card-20(22)-enolide (2) Colorless microcrystals, mp 167—171 °C (acetone–hexane); $[\alpha]_D^{20}$ −6.06 (c=0.330, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3537, 3010, 2932, 1765, 1746; UV (MeOH) nm (log ε): 218 (4.20); ^{1}H- and ^{13}C-NMR data are shown in Table 1; HR-FAB-MS m/z 533.3113 $[M+H]^+$ (Calcd for $C_{30}H_{45}O_8$, 533.3115).

(8*R*)-3β-Hydroxy-14-oxo-15(14→8)*abeo*-5β-card-20(22)-enolide (3) Colorless prisms, mp 278—285 °C (MeOH); $[\alpha]_D^{20}$ +49.60 (c=0.254, MeOH); IR (KBr) cm^{-1}: 3399, 2937, 1748, 1692; UV (MeOH) nm (log ε): 207 (4.32); ^{1}H- and ^{13}C-NMR data are shown in Table 1; HR-FAB-MS m/z 373.2376 $[M+H]^+$ (Calcd for $C_{23}H_{33}O_4$, 373.2378).

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References and Notes

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23) The $[\alpha]_D$ value of cardenolide B-2 (**2**) was evaluated as following. The observed $[\alpha]_D$ values of digitoxigenin (**16**)[18] and odoroside A (**17**)[18] are +40.3 and +1.5, respectively. From these values, the contribution of diginosyl moity to $[\alpha]_D$ values of **17** is estimated to be −38.8. The $[\alpha]_D$ value of **2** was calculated to be −24.7 based on the observed and calculated $[\alpha]_D$ values of tannigenin (**14**)[24,25] and diginosyl moity, +14.1 and −38.8, respectively.
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