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A NEW CARDENOLIDE TRIGLYCOSIDE FROM STEMS AND TWIGS OF *NERIUM OLEANDER*

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Abstract – A new cardenolide triglycoside, cardenolide B-3 (**1**) was isolated from *Nerium oleander* L. The structure of **1** was established to be 3*β*-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-diginopyranosyl]-7*β*,8-epoxy-14-hydroxy-5*β*,14*β*-card-20(22)-enolide on the basis of their spectroscopic data.

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. Cardenolides in the leaves,[1–8] roots, and root bark[9–11] of this plant were investigated because of the interests in their biological activities.[12] The cardiac glycoside digitoxin and digoxin have been used in treatment of cardiac diseases for many years.[12,13] Anticancer utilization of digitoixin, digoxin, and related cardenolides has been also investigated.[14,15] We recently reinvestigated the bioactive cardenolide monoglycosides[16] and pregnanes[17] of *N. oleander* from the interest of their biological activities. Further investigation on its chemical constitutes in more polar fraction resulted in the isolation of a new cardenolide triglycoside.

A methanol extract of air-dried stems and twigs of *N. oleander* was partitioned successively with hexane,

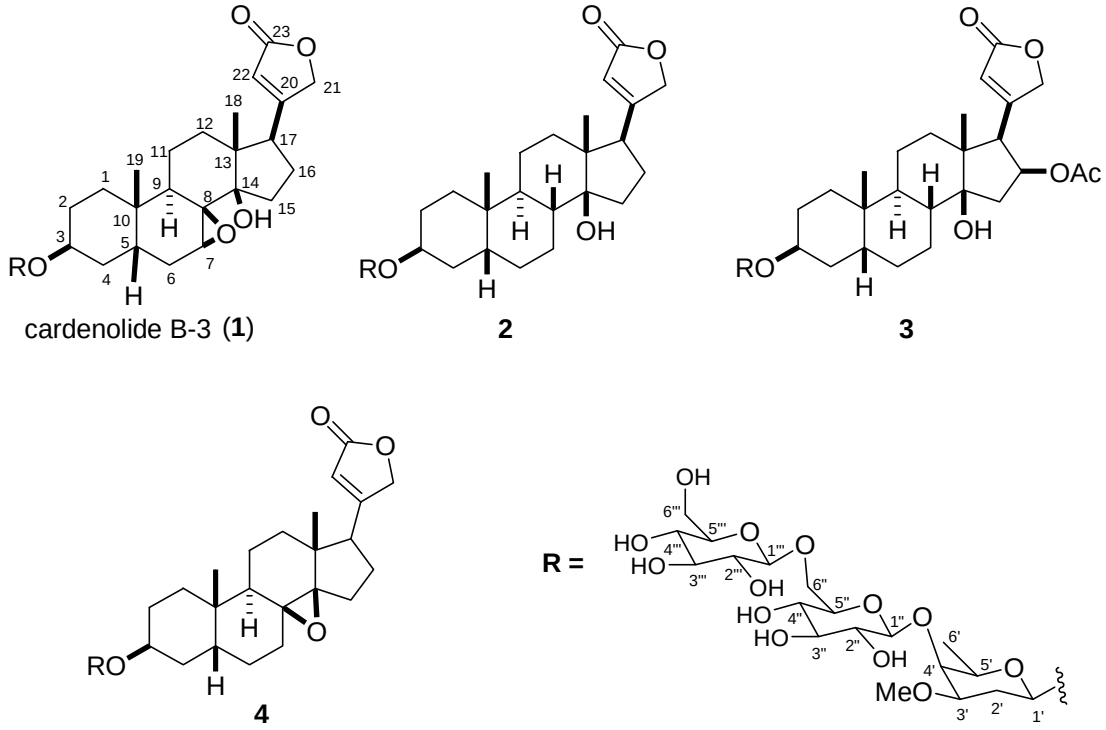

Figure 1

EtOAc, and *n*-BuOH. The *n*-BuOH extract gave a new cardenolide triglycoside, cardenolide B-3 (**1**) (Figure 1). Cardenolide B-3 (**1**) gave the elemental composition, $C_{42}H_{64}O_{18}$, which was determined by HRFABMS analysis. The IR spectrum of **1** indicated the presence of hydroxyl (3389 cm^{-1}) and α,β-unsaturated-γ-lactone (1745 and 1642 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 42 carbon signals. A carbonyl carbon resonated at δ 174.4 and two olefin carbon resonances were located at δ 175.1 (s) and 117.8 (d). Five resonances for carbons bearing oxygen were observed at δ 81.8 (s), 73.7 (t), 72.4 (d), 64.5 (s), and 51.1 (d) in addition to one methoxy methyl and sixteen oxygenated carbon signals of one 2,6-dideoxyhexose sugar and two hexose sugar moieties. From the DEPT and HMQC spectra, the remaining carbon resonances were three methyl, nine methylene, three methine, and two quaternary carbons. The ^1H NMR spectrum showed two methyl singlets (δ 1.05 and 1.06) and one additional methyl doublet of 2,6-dideoxyhexose sugar at δ 1.66 (d, J = 6.4 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 ^1H-^1H COSY spectrum. An HMBC experiment was used to determine the carbon-carbon connection through the nonprotonated carbon atoms [HMBC correlations: H-17 (δ 2.80) to C-12, C-13 (δ 52.7, s), C-14 (δ 81.8, s), C-15, C-16, C-18, C-20 (δ 175.1, s), C-21 (δ 73.7, t), and C-22 (δ 117.8, d); H-18 (δ 1.05) to C-12,

C-13, C-14 (δ 81.8, s), and C-17; H-19 (δ 1.06) to C-1, C-5, C-9, and C-10 (δ 34.0, s)]. Interpretation of these results suggests that compound **1** has steroid A, B, C, and D rings[18], bearing an α,β-unsaturated

Table 1. ^{13}C NMR Spectroscopic Data (125 MHz, C_5H_5N) of Compounds **1**, **2**, **3**, and **4**[a]

	1	**2**	**3**	**4**
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	31.9 (t)	30.7 (t)	30.7 (t)	31.0 (t)
2	27.6 (t)	27.0 (t)	27.1 (t)	27.4 (t)
3	72.4 (d)	73.0 (d)	73.6 (d)	73.6 (d)
4	33.1 (t)	30.3 (t)	30.5 (t)	30.3 (t)
5	32.0 (d)	35.8 (d)	37.0 (d)	36.7 (d)
6	28.4 (t)	27.3 (t)	27.0 (t)	27.3 (t)
7	51.1 (d)	22.0 (t)	21.7 (t)	25.2 (t)
8	64.5 (s)	41.8 (d)	42.0 (d)	65.2 (s)
9	34.4 (d)	37.0 (d)	35.9 (d)	37.1 (d)
10	34.0 (s)	35.5 (s)	35.5 (s)	37.1 (s)
11	21.0 (t)	21.5 (t)	21.2 (t)	16.5 (t)
12	40.9 (t)	39.8 (t)	39.0 (t)	36.7 (t)
13	52.7 (s)	50.1 (s)	50.5 (s)	41.8 (s)
14	81.8 (s)	84.6 (s)	83.5 (s)	70.8 (s)
15	35.4 (t)	33.1 (t)	41.3 (t)	26.9 (t)
16	28.8 (t)	27.2 (t)	75.0 (d)	25.9 (t)
17	51.0 (d)	51.4 (d)	56.8 (d)	51.4 (d)
18	17.4 (q)	16.2 (q)	16.3 (q)	16.3 (q)
19	24.5 (q)	23.9 (q)	23.9 (q)	25.0 (q)
20	175.1 (s)	176.0 (s)	170.2 (s)	170.6 (s)
21	73.7 (t)	73.7 (t)	76.2 (t)	73.6 (t)
22	117.8 (d)	117.6 (d)	121.6 (d)	116.9 (t)
23	174.4 (s)	174.5 (s)	174.1 (s)	173.8 (s)
OAc			170.0 (s), 20.7 (q)	
1'	98.9 (d)	98.7 (d)	98.9 (d)	98.8 (d)
2'	33.3 (t)	33.2 (t)	33.3 (t)	33.3 (t)
3'	80.2 (d)	80.1 (d)	80.2(d)	80.2 (d)
4'	73.6 (d)	73.2 (d)	73.1(d)	72.8 (d)
5'	71.0 (d)	70.8 (d)	70.9 (d)	70.9 (d)
6'	18.2 (q)	18.1 (q)	18.1 (q)	18.1 (q)
OMe	56.2 (q)	56.1(q)	56.2 (q)	56.2 (q)
1"	104.7 (d)	104.5 (d)	104.6 (d)	104.7 (d)
2"	75.8 (d)	75.6 (d)	75.7 (d)	75.7 (d)
3"	78.4 (d)	78.2 (d)	78.5 (d)	78.3 (d)
4"	72.0 (d)	71.8 (d)	71.8 (d)	71.9 (d)
5"	77.7 (d)	77.6 (d)	77.6 (d)	77.6 (d)
6"	70.5 (t)	70.3 (t)	70.5 (t)	70.5 (t)
1"'	105.6 (d)	105.5 (d)	105.6 (d)	105.6 (d)
2"'	75.3 (d)	75.1 (d)	75.2 (d)	75.2 (d)
3"'	78.6 (d)	78.4 (d)	78.4 (d)	78.5 (d)
4"'	71.9 (d)	71.6 (d)	71.9 (d)	71.8 (d)
5"'	78.5 (d)	78.4 (d)	78.3 (d)	78.4 (d)
6"'	62.9 (t)	62.7 (t)	62.8 (t)	62.8 (t)

[a]Signals were assigned from ^{1}H-^{1}H COSY, HMQC, and HMBC spectra.

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 sugar portion of **1** were assigned as 3-*O*-β-glucopyranosyl-

(1→6)-*β*-glucopyranosyl-(1→4)-*β*-diginopyranoside on the basis of comparisons of the ^{13}C NMR data (Table 1) with those of known analogous compounds **2**,[5,19] **3**,[5,20] and **4**[5,19] (Figure **1**).

These assignments were supported by NOESY correlations [(H-1''' with H-6'', H-3''', and H-5'''; H-2''' with H-4'''; H-3''' with H-1''' and H-5'''; H-4''' with H-2'''; H-5''' with H-1''' and H-3'''); (H-1'' with H-3'' and H-5''; H-2'' with H-4''; H-3'' with H-1'' and H-5''; H-4'' with H-2''; H-5'' with H-1'' and H-3''); (H-1' with H-3', H-5', and H-3; H-3' with H-1' and H-5'; H-4' with H-3', H-5', and OMe; H-5' with H-1', H-3', and CH_3-6') as well as coupling constants observed [H-1''' (d, *J* = 7.6 Hz); H-1'' (d, *J* = 7.8 Hz), H-1' (d, *J* = 8.3 Hz)]. The C-1'''– C-6'' linkage of glucose-glucose and the C-1''-C-4' linkage of glucose-diginose were determined by the analysis of HMBC correlations [(H-1''' to C-6'' and H-6'' to C-1''') and (H-1'' to C-4' and H-4' and C-1''). Since only D-glucose and D-diginose are known in *N. oleander*, the sugar portion in **1** was assigned as 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-diginopyranoside. 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* 3.42) with C-6, C-8 (64.5, s), C-14 (81.8, s); H-6β (*δ* 2.29) with C-4, C-5, C-7 (*δ* 51.1, d) and C-8]. The observed coupling constants of H-7 [H-7 with H-6*α* (*J* = 5.9 Hz) and H-7 with H-6*β* (*J* = 0 Hz)] are in good accordance with those deduced from the dihedral angles of H-7 with H-6*α* and H-6*β*. The *β*-orientation of the 7,8-epoxide ring was also supported by NOE correlation [H-7 with H-15*β*, H-6*α*, and the proton of 14-OH (*δ* 2.37)]. The observed NOE correlation of the proton of 14-OH 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 7,8-eopxide ring. The *β*-orientation of 14-OH was also supported by NOE correlation [H-12*α* with H-9 and H-15*α*] that indicated *cis*-ring junction of C and D ring. Thus, 7,8-epoxide ring, 14-hydroxyl group, and CH_3-18 are located in *cis*-*β*-orientation. The analysis of NOESY correlations [CH_3-19 with H-1*β*, H-5, H-6*β*, and 11*β*; H-9 with H-2*α*, H-4*α*, H-11*α*, and H-12*α*; H-12*α* with H-17; CH_3-18 with 12*β*, H-21, and H-22] indicated the relative stereochemistry of **1** to be 3*β*-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-diginopyranosyl]-7*β*,8-epoxy-14-hydroxy-5*β*,14*β*-card-20(22)-enolide. Since all known cardenolides isolated from *N. oleander* possess same absolute configuration in genin moiety, the absolute configuration of **1** is regarded as 3*S*, 5*S*, 7*S*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*, and 17*R*.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined by Yanagimoto micro-melting point instrument and 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. HRFABMS were recorded on a JEOL JMS-HX110 instrument. Silica gel (70－230 mesh) was employed for column chromatography. HPLC separations were performed on a Hitachi L-6200 HPLC instrument 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. The air-dried stems and twigs (1.95 kg) were combined and extracted with MeOH (8.5 L) for 20 days. The MeOH extract was concentrated to 400 mL and extracted with hexane (8 × 100 mL). Water (130 mL) was added to the MeOH layer, extracted with EtOAc (3 × 300 mL). Aqueous layer was further extracted with *n*-butanol, dried (Na_2SO_4), and concentrated to give an oily materal (24.4 g). This was separated by column chromatography [silica gel (2.1 kg), a gradient of $CHCl_3$ and MeOH] into fourteen fractions, A–N. Fraction F [$CHCl_3$–MeOH (8:2)] gave on drying viscous oils, weighing 4.21 g, which was further separated by HPLC [ODS, MeOH–MeCN-H_2O (1:4:10)] to give six fractions, F1–F6. F2 gave on drying viscous oil, weighing 282 mg, which was subjected to HPLC [ODS, MeCN-H_2O (3:11)] to give five fractions, F21–F25. F23 afforded on drying compound **1** [16.2 mg (0.00083%)]. Considering separation conditions mentioned above, there is no possibility that **1** is artifact. Actually, **1** was detected by the analyses of HPLC [ODS, MeOH-MeCN-H_2O (1:4:10)] in BuOH extract.

Cardenolide B-3 (**1**)**:** colorless microcrystals; mp 169–171 °C (acetone–hexane); $[\alpha]^{20}_{D}$ –1.62˚ (*c* 0.308, MeOH); ^{1}H NMR (C_5D_5N) δ 6.11 (1H, br s, H-22), 5.17 (1H, dd, *J* = 17.8, 1.7 Hz, H-21b), 5.15 (1H, d, *J* = 7.6 Hz, H-1'''), 5.09 (1H, d, *J* = 7.8 Hz, H-1''), 4.97 (1H, dd, *J* = 17.8, 1.2 Hz, H-21a), 4.80 (1H, br dd, *J* = 11.7, 2.5 Hz, C-6b''), 4.64 (1H, br dd, *J* = 8.3 Hz, H-1'), 4.50 (1H, *J* = 11.8, 5.4 Hz, H6b'''), 4.35 (1H, *J* = 11.8, 2.4 Hz, H6a'''), 4.31 (1H, m, H-4'), 4.27 (1H, dd, *J* = 11.7, 6.8 Hz, C-6a''), 4.21 (1H, m, H-4'''), 4.20 (1H, br s $W_{h/2}$ = 7.5 Hz, H-3), 4.19 (1H, m, H-3'''), 4.12 (1H, dd, *J* = 9.0, 9.0 Hz, H-3''), 4.06 (1H, m, H-5''), 4.02 (1H, m, H-2'''), 3.97 (1H, m, H-4''), 3.92 (1H, m, H-5'''), 3.88 (1H, m, H-2''), 3.52 (1H, q, *J* = 6.4 Hz, H-5'), 3.42 (1H, d, *J* = 5.9 Hz, H-7), 3.39 (1H, m, H-3'), 3.35 (3H, s, OCH_3), 2.80 (1H, dd, *J* = 5.6, 4.6 Hz, H-17), 2.40 (1H, m, H-15a), 2.37 (14-OH), 2.36 (1H, m, H-9), 2.32 (1H, m, H-2'b), 2.29 (1H, m, H-6b), 2.19 (1H, m, H-16a), 2.09 (1H, m, H-2'a), 2.04 (1H, m, H-16b), 1.90 (1H, m, H-2b), 1.86 (1H, m, H-15b), 1.74 (1H, m, H-5b), 1.69 (1H, m, H-2a), 1.66 (1H, d, *J* = 6.4 Hz, H-6'), 1.63 (1H, m, H-12b), 1.60 (1H, m, H-4b), 1.56 (1H, m, H-12a), 1.53 (1H, m, H-1a), 1.50 (1H, m, H-4a), 1.48 (1H, m, H-6a),

1.45 (1H, m, H-1b), 1.06 (3H, s, CH_3-19), 1.05 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 175.1 (C-20), 174.4 (C-23), 117.8 (C-22), 105.6 (C-1'''), 104.7 (C-1''), 98.9 (C-1'), 81.8 (C-14), 80.2 (C-3'), 78.6 (C-3'''), 78.5 (C-5'''), 78.4 (C-3''), 77.7 (C-5''), 75.8 (C-2''), 75.3 (C-2'''), 73.7 (C-21), 73.6 (C-4'), 72.4 (C-3), 72.0 (C-4''), 71.9 (C-4'''), 71.0 (C-5'), 70.5 (C-6''), 64.5 (C-8), 62,9 (C-6'''), 56.2 (OMe at C-3'), 52.7 (C-13), 51.1 (C-7), 51.0 (C-17), 40.9 (C-12), 35.4 (C-15), 34.4 (C-9), 34.0 (C-10), 33.3 (C-2'), 33.1 (C-4), 32.0 (C-5), 31.9 (C-1), 28.8 (C-16), 28.4 (C-6), 27.6 (C-2), 24.5 (C-19), 21.0 (C-11), 18.2 (C-6'), 17.4 (C-18); IR (KBr) ν_{max} 3389, 2973, 1745, 1642, 1100, 1028 cm^{-1}; UV (MeOH) λ_{max}(log ε): 216 (4.05) nm; CD (MeOH) $[\theta]_{289}$ –900, $[\theta]_{238}$ +9170; HRFABMS *m/z* 855.4024 (calcd for $C_{42}H_{63}O_{18}$ $[M - H]^-$, 855.4014).

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