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CARDIAC GLYCOSIDES OF THE ROOT BARK OF *NERIUM ODORUM**

TATSUO YAMAUCHI, MICHIKO TAKAHASHI and FUMIKO ABE
Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

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Key Word Index—*Nerium odorum*; Apocynaceae; oleander; cardiac glycosides; cardenolides; uzarigenin glycosides; oleandrigenin glycosides.

Abstract—In addition to the known odorosides, the β-D-digitaloside and β-D-glucosyl-(1 → 4)-β-D-digitaloside of uzarigenin were isolated from the root bark of *Nerium odorum*. Odoroside B was obtained in remarkably high yield among the digitoxigenin and uzarigenin glycosides. With the aid of polyamide column chromatography, oleandrigenin β-gentiobiosyl-(1 → 4)-β-D-digitaloside (= 16-*O*-acetylneogitostin) was isolated along with other oleandrigenin glycosides.

INTRODUCTION

Reichstein and his associates [1] surveyed cardiac glycosides in the stem bark of *Nerium odorum* grown in India, and found many glycosides including odoroside A, B, D, G, H, K, bioside K, graciloside, and a mixture of 16-*O*-acetyl- and 16-anhydro-digitalinum verum (Table 1). Present authors previously reported on acetophenones [2], pregnenolone glucosides [3], and pregnane [4] in the root bark, and cardiac glycosides in oven-dried [5] and air-dried leaves [6]. This paper deals with the cardiac glycosides including three new uzarigenin and oleandrigenin glycosides in the root bark, and the comparison of the digitoxigenin and uzarigenin glycosides with those from the stem bark.

* Part 6 in the series "*Nerium*". For Part 5 see [6].

† $[M]_D$ values of α- and β-methyl D-digitaloside were reported as +243° and −5°, respectively by Rheiner *et al.* [1].

RESULTS

The methanol percolate of powdered root bark was diluted with water and partitioned with hexane, benzene, $CHCl_3$, and with BuOH, successively. The $CHCl_3$ layer was again treated with 50%-MeOH and fractionated to $CHCl_3$ (C-1) and aqueous (C-2) layers. A part of each fraction was submitted to silica gel (Si) column chromatography, using benzene–acetone or the lower layer of $CHCl_3$–MeOH–water as eluant. From these fractions two new glycosides, **1** and **2** were isolated, in addition to the known digitoxigenin and uzarigenin glycosides, odoroside A, B, G, H, K, and bioside K (Tables 1, 2).

Compound **1**, $C_{30}H_{46}O_8$, isolated from benzene and C-1 fractions, was hydrolyzed to uzarigenin and D-digitalose. The configuration of the linkage of digitalose to uzarigenin was shown as β by the coupling constant of anomeric proton (*d*, $J = 8$ Hz), and by $[M]_D$ difference ($\Delta M_D = +75°$)† of **1** and uzarigenin. Compd. **1** afforded two monoacetates **1A-I** (mp 234°) and **1A-II** (mp 200°),

Table 1. Cardiac glycosides of the root bark of *Nerium adorum*

Sugars	Aglycones		
	Digitoxigenin	Uzarigenin	Oleandrigenin
β-D-Diginoside	Odoroside A*	Od. B*	Nerigoside†
β-D-Digitaloside	Od. H*	Compd. **1**	Neritaloside†
β-D-Glucosyl-β-D-Diginoside	Od. D*	Bioside K*	Glucosyl-nerigoside‡
β-D-Glucosyl-(1 → 4)-β-D-Digitaloside	(Bioside G)‡	Compd. **2**	Glucosyl-neritaloside§*
β-Gentiobiosyl-β-D-Diginoside	—	Od. K*	—
β-Gentiobiosyl-(1 → 4)-β-D-Digitaloside	Od. G*	—	Compd. **3** (16-*O*-acetyl-neogitostin)

* Obtained from stem bark[1]. † Obtained from seeds [7]. ‡ Obtained by enzymatic hydrolysis of Od. G [1]. § Obtained from air-dried leaves [6].

Table 2. Yields of the digitoxigenin and uzarigenin glycosides (%)

	Digitoxigenin glycosides			Uzarigenin glycosides				
	Od. A	Od. H	Od. G	Od. B	Biosid. 1	Biosid. K	Biosid. 2	Od. K
Root bark	0.070	0.043	0.040	0.450	0.054	0.049	0.021	0.079
Stem bark	0.072	0.115	0.108	0.130	0.020	0.001	0.016	0.091

on limited acetylation or on mild saponification of **1**-diacetate, and in order to verify the position of acetyl residue, each monoacetate was compared with odoroside H monoacetate on NMR spectroscopy (Table 3). For odoroside H monoacetate, double–doublet signals at δ 5.16 (J = 8 and 10 Hz) and at δ 3.92 (J = 1 and 4 Hz) were assigned respectively to 2′ and 4′ protons according to their coupling constants and the spin decoupling procedure of 1′, 2′, 3′ (δ 3.34, *dd*: J = 4 and 8 Hz), and 4′ protons. A similar double–doublet resonance with small coupling (like that at δ 3.92) was given in **1A-I** at δ 3.68 rather than in **1A-II**, whose corresponding proton was observed at δ 5.02 with a downfield shift by acetylation of the hydroxyl, and another *dd* line at 3.56 with a splitting of 6 and 10 Hz. **1A-I** and **1A-II** were thus assigned as the 2′-*O*- and 4′-*O*-acetate, respectively.

Compound **2**, $C_{36}H_{56}O_{13}$, was obtained from C-2 and BuOH fractions and, on acid hydrolysis, afforded uzarigenin, D-digitalose and D-glucose. Although partial hydrolysis of **2**–**1** was unsuccessful, MS spectroscopy of **2**-pentaacetate exhibited a peak at *m/e* 331, ascribable to the terminal acetylated glucose residue. Thus, glucose is linked to digitalose. The pentaacetate was saponified to give a monoacetate. Since a double–doublet of the **2**-monoacetate at δ 5.82 coincided with those of bioside G monoacetate (δ5.84) and of odoroside H monoacetate (δ 5.89) (Table 3), their acetyl residues were at 2′-OH, and consequently the glucose of **2**, as well as that of bioside G, was attached to 4′-OH of digitalose. The β-configuration of glucose residue was supported by $[M]_D$ difference of **2** and **1** ($\Delta M_D = -61°$). Hence, the structure of **2** was deduced to be uzarigenin β-D-glucosyl-(1 → 4)-β-D-digitaloside.

In order to compare the cardiac glycosides of the bark in aerial and underground parts, the major glycosides were isolated from the stem bark by the same procedure as used for the root bark (Table 2).

When polyamide (Po) column chromatography was carried out prior to Si column chromatography, the oleandrigenin glycosides in each fraction (nerigoside, neritaloside, glucosylnerigoside or glucosyl-neritaloside) were eluted before digitoxigenin and uzarigenin glycosides. In addition to the known oleandrigenin glycosides, Compd. **3**, showing the same fluorescence as the oleandrigenin glycosides with the $SbCl_3$ reagent under UV light, was isolated from BuOH fraction, along with glucosylneritaloside. On enzymatic hydrolysis, **3** furnished digitalinum verum and its monoacetate, whose aglycone was confirmed as oleandrigenin on hydrolysis. Compound **3** appeared to be composed of oleandrigenin, D-digitalose and possibly two moles of D-glucose. The acetyl residue of **3** was removed by alkali to provide a more polar non-crystalline glycoside which was further hydrolyzed to digitalinum verum. Gentiobiose α-acetate was obtained on acetolysis of **3**-acetate. These results suggest that **3** is oleandrigenin β-gentiobiosyl-(1 → 4)-β-D-digitaloside (= 16-*O*-acetylneogitostin) and, in fact, the physical constants of **3**-acetate are in good agreement with

Table 3. NMR of the acetylated digitalosides and glucosyldigitalosides of digitoxigenin and uzarigenin (ppm)

Glycosides	Solvent*	1′-H	2′-H	4′-H	22-H
Uzarigenin digitaloside 2′-*O*-acetate (**1A-I**)	C-M	4.21 (*d*, J = 8 Hz)	4.6 ~ 4.9‡	3.68 (*dd*, J = 1, 3 Hz)	5.60
Uzarigenin digitaloside 4′-*O*-acetate (**1A-II**)	C-M	4.16 (*d*, J = 8 Hz)	3.56 (*dd*, J = 6, 10 Hz)	5.02 (*dd*, J = 1, 3 Hz)	5.59
Uzarigenin digitaloside 2′,4′-diacetate	C	4.48 (*d*, J = 8 Hz)	5.01 (*dd*, J = 8, 10 Hz)	5.32 (*dd*, J = 1, 4 Hz)	5.89
Odoroside H 2′-*O*-acetate	C	4.21 (*d*, J = 8 Hz)	5.16 (*dd*, J = 8, 10 Hz)	3.92 (*dd*, J = 1, 4 Hz)	5.98
	P	4.80 (*d*, J = 8 Hz)	5.89 (*dd*, J = 8, 10 Hz)	4.15 (*dd*, J = 1, 4 Hz)	6.20
Odoroside H 2′,4′-diacetate	C	4.44 (*d*, J = 8 Hz)	5.10 (*dd*, J = 8, 10 Hz)	5.37 (*dd*, J = 1, 4 Hz)	5.92
Uzarigenin glucosyl digitaloside monoacetate (**2MA**)	C-M			3.96 (*dd*, J = 1, 4 Hz)	5.60
	P		5.82 (*dd*, J = 8, 10 Hz)		6.13
Odorobioside G Monoacetate	P		5.84 (*dd*, J = 8, 10 Hz)		6.24

* C: $CDCl_3$, M: CD_3OD, C-M: 1:1 mixture of C and M, P: C_5D_5N. † In order to measure the anomeric proton, trifluoroacetic acid was added [8]. ‡ Overlapped with 21-methylene signal.

the data already reported on neogitostin nonaacetate by Okano [9].

DISCUSSION

The digitaloside (**1**) and glucosyldigitaloside (**2**) of uzarigenin were obtained from the stem bark of the plants we examined, whereas neither of these two glycosides were present in the stem bark of *N. odorum* grown in India [1]. On the other hand, the latter contained graciloside which was not found in the Japanese plant. This is obviously yet another example of chemical races. Comparison of the digitoxigenin and uzarigenin glycosides in the root and stem barks showed the predominancy of uzarigenin glycosides, especially, odoroside B in the former.

The sugar moieties of odorobioside G and graciloside were believed respectively as glucosyl-(1 → 4) and -(1 → 2)-digitalose, but no positive proof has been given. In this study, the 1 → 4 linkage of glucose to digitalose in both **2** and bioside G were confirmed by the aid of NMR spectroscopy.

Separation of the cardenolides having same sugar moieties, such as gentiobiosyldigitaloside of oleandrigenin and of digitoxigenin, or glucosyldigitaloside of oleandrigenin and of uzarigenin have been not completely successful using Si column chromatography. We have shown that if the crude mixture is first passed through polyamide column, oleandrigenin glycosides can be eluted out with water prior to those of digitoxigenin or uzarigenin, and each of the minor oleandrigenin glycosides can then be isolated satisfactory with the aid of Si column chromatography.

EXPERIMENTAL

Abbreviation. Following abbreviations were used; odorosides A, B, ~ K: A, B, ~ K, bioside K: BK, nerigoside; Ng, neritaloside: Nt, strospeside: SS, glucosylnerigoside: GNg, glucosylneritaloside (= 16-*O*-acetyl digitalinum verum): GNt, pregnenolone *β*-glucosyl-(1 → 2)-*β*-glucoside: P3, pregnenolone *β*-glucosyl-(1 → 6)-*β*-glucoside: P2, pregnenolone bis-*β*-glucosyl-(1 → 2, 1 → 6)-*β*-glucoside: P1, yellow pigments: Y9 and Y6.

General procedure. Mp's were measured on Kofler block and uncorr. Optical rotations and UV absorptions were measured in MeOH at 20°, and at room temp., respectively. TLC and PC were conducted principally in the same manner described in the preceding paper [6].

Extraction and fractionation. Root bark (10 kg) was powdered and percolated with MeOH and the concentrate of percolates diluted with H_2O (1:1). The mixture was partitioned with hexane, C_6H_6 (ext. 150 g), $CHCl_3$ (227 g), and BuOH (162 g), successively. The $CHCl_3$ ext. was again dissolved in $CHCl_3$, back extracted with 50%-MeOH, and both $CHCl_3$ (C-1) and aq (C-2) layers were concentrated (100 g and 110 g, respectively). Each fraction was subjected to Si column chromatography with C_6H_6–Me_2CO (for C_6H_6 fraction) or $CHCl_3$–MeOH–H_2O (7:2:1.2 ~ 0.7, 7:3:1.2 ~ 0.7) and the fractions containing each glycoside were crystallized to afford pure samples. The bark of stem was worked up in the same way. The overall yields were listed in Table 2.

Uzarigenin β-D-digitaloside (Compd. **1**). Compound **1** was isolated from C_6H_6 and C-1 fractions by Si column chromatography with C_6H_6–Me_2CO elution (4:1) and crystallized from hexane–EtOAc to give needles, mp 239–240°, $[\alpha]_D$ + 4.2°, ($[M]_D$ + 22.5°; $[M]_{D.1}$–$[M]_{D.uzarigenin}$ = + 75°), UV λ_{max} 218 nm (ϵ 15900), (Found: C, 67.3; H, 8.7. $C_{30}H_{46}O_8$ requires C, 67.4; H, 8.7%). *Compd.* **1** *diacetate.* mp 262–264°, $[\alpha]_D$ + 66.7° (*c* 0.12), (C, 64.4; H, 8.1. $C_{34}H_{50}O_{10}\cdot H_2O$ requires C, 64.1; H, 8.2%). Monoacetates of **1** (**1A-I**) and (**1A-II**) were obtained by partial acetylation of **1** with eq Ac_2O in C_5H_5N (**1A-I**) or saponification of the diacetate with $KHCO_3$, followed by separation of the products by Si column chromatography. *1A-I.* mp 233–234°, $[\alpha]_D$ + 15.0° (*c* 0.20). *1A-II* mp 197–200°, $[\alpha]_D$ −8.0° (*c* 0.15). On reflux of **1** (150 mg) with 20 ml of 0.1 N-H_2SO_4–50% EtOH for 3 hr, anhydrouzarigenin (mp 270–274°) and uzarigenin (mp 248–251°) were obtained and identified by direct comparison with authentic samples (mmp, IR). D-digitalose was detected from H_2O layer of the hydrolyzate (PC: R_f 0.39 in. toluene–BuOH (1:9) satd. with H_2O; specimen D-diginose 0.69; D-digitalose 0.39, D-glucose 0.07: R_f 0.49 in. BuOH–AcOH–H_2O (4:1:5); D-digitalose 0.48, D-glucose 0.16).

Uzarigenin β-D-glucosyl-(1 → 4)-β-D-digitaloside (Compd. **2**). C-1 and C-2 fractions were chromatographed on Si column with $CHCl_3$–MeOH–H_2O (7:2:1) and the fractions containing **2** were crystallized from MeOH to give prisms, mp 315–325°, $[\alpha]_D$ −12.0° (*c* 0.08), ($[M]_D$ −83.6°; $[M]_{D.2}$–$[M]_{D.1}$ = −61°), UV λ_{max} 218 nm (ϵ 17900), (Found: C, 60.2; H, 8.1. $C_{36}H_{56}O_{13}\cdot H_2O$ requires C, 60.5; H, 8.2%). *Compd.* **2** *pentaacetate.* mp 270–274°, $[\alpha]_D$ −10.9° (*c* 0.11), (Found: C, 60.7; H, 7.2. $C_{46}H_{66}O_{18}$ requires C, 60.9; H, 7.3%). MS: *m/e* 331 (peracetylated terminal hexose residue). *Compd.* **2** *monoacetate* (**2MA**). **2MA** was prepared by saponification of the pentaacetate with $KHCO_3$, followed by crystallization from MeOH to give needles, mp 255–260°, $[\alpha]_D$ −3.6° (*c* 0.11). Compd. **2** (300 mg) was refluxed with 0.1 N-H_2SO_4–50% EtOH for 2 hr to give a small amount of uzarigenin (mp 241–245°) identified by direct comparison with authentic sample (mmp, IR). D-digitalose and D-glucose (PC: R_f Tol-B 0.39, 0.07; BAW, 0.49, 0.17) were detected from the water layer of hydrolyzate.

Isolation of the oleandrigenin glycosides using polyamide column. C_6H_6 and C-1 extracts were mixed with Celite respectively and placed on a top of polyamide column (polyamide: 50 × wt of sample) and eluted with H_2O and then with MeOH–H_2O. C-2 and BuOH extracts were dissolved in H_2O, passed through column and eluted with H_2O. C_6H_6 fraction (15 g) was fractionated to f-1 (0.2 g; Nt, Ng). f-2 (0.8 g; H, **1**, Ng), f-3 (0.9 g; H, **1**, A, B) and f-4 (5.5 g; A, B). C-1 (18 g) was fractionated to f-1′ (3.7 g; P1, K, GNt, GNg, SS, Nt), f-2′ (1.3 g; P1, K, **2**, BK, H, Nt), f-3′ (1.3 g; P1, P2, **1**, H) and f-4′ (1.7 g; P1, P2, **1**). C-2 (27 g) was fractionated to f-1″ (2.5 g; sugars), f-2″ 2.2 g; sugars, **3**, GNt), f-3″ (0.5 g; **3**, G), f-4″ (10.7 g; G, K, P1, P2, P3), f-5″ (3.2 g; P2, P3, Y9) and f-6″ (3.0 g; P3, Y9, Y6). BuOH fraction (30 g) was fractionated to f-1‴ (3.1 g; sugars), f-2‴ (3.1 g; sugars, **3**, GNt), f-3‴ (3.4 g; G, K), f-4‴ (4.1 g; P1, P2, P3) and f-5‴ (0.8 g; Y9, Y6). On Si column chromatography of f-1′, followed by crystallization from dioxane–Et_2O–hexane, Ng was obtained as needles, mp 165–167°, $[\alpha]_D$ −16.0° (*c* 0.10) (yield from the bark: 0.003%). Nt was obtained as solid from f-1 and f-1′ (0.004%) and saponified to SS (mp 253–256°). SS was isolated from f-1′ and crystallized from MeOH to give needles, mp 256–258°, $[\alpha]_D$ + 20.0° (*c* 0.11) (0.002%). GNg was obtained from f-1′, mp 174–178°, $[\alpha]_D$ + 31.3° (*c* 0.12) (0.003%) GNt was obtained from f-1′, f-2′ and from f-2‴ as solid (0.005%).

GNt-pentaacetate. mp 184–190° ~ 205–215°, $[\alpha]_D$ −20.7° (*c* 0.06). GNt gave digitalinum verum, mp 240–243°, on $KHCO_3$ saponification, and oleandrigenin (mp 230–233°) on acid hydrolysis.

Oleandrigenin β-gentiobiosyl-(1 → 4)-β-D-digitaloside (= 16-*O-acetyl neogitostin*) (**3**). f-2‴ and f-2″ were chromatographed on Si with $CHCl_3$–MeOH–H_2O (7:2:0.8 ~ 0.6) and then with EtOAc–MeOH (10:1 ~ 7.5:1) and **3** was obtained as homogeneous solid, 70 mg. *Compd.* **3** *nonaacetate.* mp 198–199°, $[\alpha]_D$ −27.0° (*c* 0.37) (neogitostin acetate [9]: mp 194–197°, $[\alpha]_D$ −32.5°). UV λ_{max} 218 nm (ϵ 20000), (Found: C, 57.1; H, 6.8. $C_{60}H_{84}O_{28}$ requires C, 57.5; H, 6.8%).

Compd. **3** *monoacetate.* On saponification of **3**-nonaacetate with $KHCO_3$ followed by crystallization from MeOH, needles were obtained, mp 253–257°, $[\alpha]_D$ −16.0°) (*c* 0.10) (neogitostin

monoacetate: mp 249–252°. $[\alpha]_D$ −14.2° in MeOH). Compd. **3** (100 mg) was dissolved in water and treated with β-glucosidase to give digitalinum verum (10 mg, mp 245–248°) and its acetate (10 mg), the latter of which was superimposable on TLC with GNt, and hydrolyzed under the Mannich's condition followed by purification to give oleandrigenin (mp 230–233°, mmp, IR). D-digitalose and D-glucose (PC: R_f Tol-B, 0.38, 0.07; BAW 0.49, 0.16). Compd. **3** nonaacetate (20 mg) was heated at 100° with 2 ml of Ac_2O and trace of $ZnCl_2$ for 30 min and poured into ice-water. α-Pentaacetyl glucose was isolated on column chromatography and crystallized from EtOH as needles (mp 190–194°, mmp). Compd. **3** (140 mg) was treated with 80 mg of $KHCO_3$ in 16 ml of 75% EtOH for 3 days. The product (80 mg) was hydrolyzed with β-glucosidase to give digitalinum verum (mp 235–240°, mmp).

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