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CARDIAC GLYCOSIDES OF THE LEAVES OF *NERIUM ODORUM**

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Key Word Index—*Nerium odorum*; Apocynaceae; oleander; cardiac glycosides; cardenolides; oleandrosides; nerigosides.

Abstract—Oleandrigenin-β-D-glucosyl-β-D-diginoside (glucosyl nerigoside) and -β-gentiobiosyl-α-L-oleandroside (gentiobiosyl oleandrin) were isolated as the major glycosides of air-dried leaves of *Nerium odorum*, along with oleandrigenin-β-D-glucoside, digitoxigenin-β-gentiobiosyl-β-D-diginoside (gentiobiosyl odoroside A), 16-*O*-acetyl-digitalinum verum, Δ^{16}-dehydroadynerigenin-D-glucosyl-β-D-digitaloside and odoroside G. Both adynerin and Δ^{16}-dehydroadynerin were also present as gentiobiosides in the leaves.

INTRODUCTION

Since oleandrin was isolated from oleander leaves and its structure established, many glycosides [1–4] including adynerin, neriantin, desacetyloleandrin, odoroside H, gitoxigenin, neritaloside, strospeside and urechitoxin have been obtained from dried leaves with or without the aid of glucosidase treatment. In 1961, Görlich [5] isolated mono- and diglucosides of oleandrin from dried leaves, regarding them as the native glycosides. The present authors previously described [6] the isolation of oleandrin in good yield, together with odoroside A, adynerin and Δ^{16}-dehydroadynerin [7], from leaves dried in the oven, while in extracts of fully air-dried leaves no oleandrin was obtained, but more polar glycosides. The identities of these compounds with those of fresh leaves were established by TLC [6]. This paper deals with the native glycosides in the leaves of *N. odorum*.

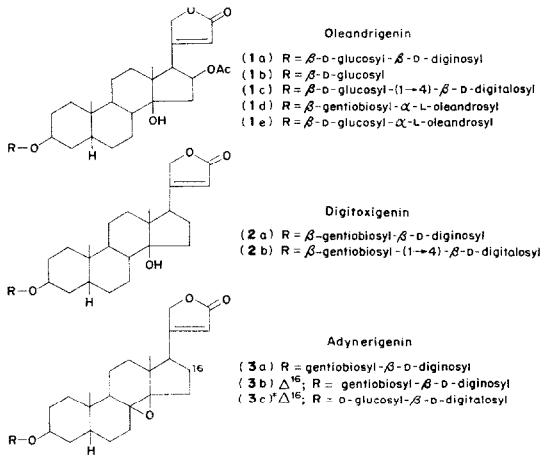
RESULTS

The methanol percolate of air-dried leaves indicated, on TLC, two major spots (**1a**) and (**1d**), which appeared to correspond to Görlich's oleandrin monoglucoside and diglucoside respectively [5]. The concentrate of the extract was partitioned with benzene (oleandrin [6]), followed by chloroform (containing **1a**) and with chloroform–ethanol (2:1) (containing **1d**), successively.

Compound **1a** (m.p. 178–181°, $[\alpha]_D$ −34·0°), obtained on crystallization from ethyl acetate–ethanol following column chromatography of the chloroform fraction, exhibited similar R_f values as monoglucosyl oleandrin (**1e**). On acid hydrolysis, however, diginose, rather than oleandrose, was detected accompanied by 1 mol of glucose, and oleandrigenin as an aglycone. Hydrolysis with snail esterase yielded needles, which were further hydrolysed to gitoxigenin and diginose, and identified as desacetylnerigoside by direct comparison with authentic sample. The β-configuration of glucose attached to diginose was supported by the molecular rotation difference (−153°) between **1a** and nerigoside. Thus, the structure of **1a** is defined as oleandrigenin β-D-glucosyl-β-D-diginoside (glucosyl nerigoside).

Along with **1a**, a small amount of **1b** was isolated and its acetate was crystallized. MS of **1b** acetate, gave peaks at *m/e* 684 and 331 showing (M^+–HOAc–H_2O) and a terminal peracetylated hexose residue, respectively. Enzymatic and acidic

* Part 5 in the series "*Nerium*". For Part 4 see Yamauchi, T., Abe, F., Ogata, Y. and Takahashi, M. (1974) *Chem. Pharm. Bull. (Tokyo)* **22**, 1680.



*The location of glucose residue on digitalose is not determined.

cleavage of **1b** and desacetyl **1b**, yielded oleandrigenin, gitoxigenin, and glucose. Compound **1b**, therefore, was oleandrigenin monoglucoside and the linkage of glucose was determined as β because of the difference of the molecular rotations ($-112°$) **1b** and oleandrigenin.

1c was obtained as an amorphous solid by chromatography of the mother liquor from the crystallization of **1a**, and gave digitalinum verum on saponification. Acid hydrolysis provided oleandrigenin, digitalose and glucose. **1c**, therefore, is considered to be 16-*O*-acetyl digitalinum verum.

In order to isolate **1d**, the $CHCl_3$–EtOH fraction was subjected to column chromatography, followed by crystallization from MeOH to yield the compound as needles. The physical constants of **1d** (m.p. 169–173°, $[\alpha]_D$ $-47{\cdot}8°$) are not completely identical with those of the oleandrin diglucoside reported by Görlich [5]. However, the fact that **1d** gave, with esterase, oleandrin monoglucoside (**1e**), and on acid hydrolysis, oleandrigenin, oleandrose and 2 mol of glucose which were in the form of gentiobiose according to the results of acetolysis of **1d** acetate, unequivocally shows its structure as oleandrigenin gentiobiosyl-α-L-oleandroside (gentiobiosyl oleandrin). The configuration of glycosidic linkage of gentiobiose to oleandrose was confirmed as β from the molecular rotation difference ($-124°$) between **1e** and oleandrin, and also by coupling constant of the anomeric proton of glucose bound to oleandrose [8].

During fractionation of **1d**, two minor glycosides, **2a** and odoroside G (**2b**) were isolated. Compound **2a**, eluted immediately following **1d** on column chromatography, was an amorphous solid and was purified through crystallization of its acetate. It afforded, on acid hydrolysis, diginose, glucose and gentiobiose along with digitoxigenin and anhydrodigitoxigenin, and by β-glucosidase, odoroside D (monoglucosyl odoroside A). On acetolysis of **2a** acetate under the usual conditions, gentiobiose peracetate was formed as in the case of **1d**. Consequently, **2a** is assigned the structure, digitoxigenin β-gentiobiosyl-β-D-diginoside (gentiobiosyl odoroside A). The final fraction of column chromatography of the $CHCl_3$ EtOH fraction afforded **2b**, and its acetate furnished a monoacetate on mild saponification. The identity of **2b** as odoroside G was confirmed on direct comparison of peracetates.

Since most of the native glycosides in this plant are gentiobiosides, it seemed possible that gentiobiosyl nerigoside was present. The $CHCl_3$ EtOH fraction, was treated with enzyme and the resultant less polar glycosides were fractionated by column chromatography. However, neither glucosyl nerigoside nor nerigoside could be detected in any fraction.

The $SbCl_3$ reagent revealed several yellow spots on the TLC of the methanol percolate, which were assumed to be the glycosides of Δ^{16}-dehydroadynerigenin [7]. One of these compounds was isolated in crystalline form from the $CHCl_3$ extract and found to be Δ^{16}-dehydroadynerigenin glucosyl digitaloside (**3c**). Another fraction was obtained during the purification of **1d** by column chromatography and, finally, an amorphous powder was obtained. This fraction was homogeneous on TLC, and on hydrolysis yielded Δ^{16}-dehydroadynerigenin and $\Delta^{5,9,14}$-anhydroadynerigenin (3β-hydroxy-5β-carda-8:9,14,20:22-trienolide) probably formed from adynerigenin [2, last ref.], together with diginose and glucose. Since the acetate of the amorphous fraction yielded gentiobiose peracetate on acetolysis, it is apparent that the original fraction contains a mixture of the gentiobiosides of adynerin (**3a**) and Δ^{16}-dehydroadynerin (**3b**).

DISCUSSION

In this study, oleandrin, odoroside A, adynerin and Δ^{16}-dehydroadynerin, previously obtained from

oven-dried leaves, were proved to present in the fresh leaves as gentiobiosides. Monoglucosyl nerigoside, rather than monoglucosyl oleandrin [5], was isolated as one of the major glycosides. Indeed, in spite of an extensive column chromatographic survey, monoglucosyl oleandrin could not be detected in the extract, and it should be noted that nerigoside is present only as its monoglucoside and not as gentiobioside in the air-dried leaves.* It should also be noted that since Δ^{16}-cardenolides are known to be artifacts [9], the true form of **3b** in the leaves remains to be explained.

EXPERIMENTAL

General procedure. Physical constants: M.ps were measured on Kofler block and uncorr. Optical rotations and UV absorptions were measured in MeOH at 20° and in EtOH at room temp, respectively. TLC: Si gel plates were developed with hexane–EtOAc (1:3), C_6H_6–Me_2CO (5:2), lower layer of $CHCl_3$–MeOH–H_2O mixture (7:3:1 ~ 7:2:1), or hexane–EtOAc–Me_2CO (0·5:1:1), and visualized by $SbCl_3$ reagent with the aid of UV-light. PC: toluene–BuOH (1:9) sated. with H_2O [Solv. 1], BuOH–AcOH–H_2O (4:1:5) [Solv. 2]. Column chromatography: Si gel (50–100 × wt of sample) columns were developed with $CHCl_3$–MeOH [Solv. 1], lower layer of $CHCl_3$–MeOH–H_2O (7:2:1·5 ~ 7:2:0·7; 7:3:1·2 ~ 7:3:0·7) [Solv. 2], EtOAc–MeOH [Solv. 3], or C_6H_6–Me_2CO [Solv. 4]. Acid hydrolysis: The samples were refluxed with 0·05 N HCl–50% MeOH for 5 min (condition 1); refluxed with 0·05 N H_2SO_4–50% MeOH for 30 min (condition 2); or dissolved in Me_2CO and allowed to stand for 1 week with a small amount of conc HCl (Mannich's condition). Enzymatic hydrolysis: Snail esterase was prepared by Me_2CO precipitation of snail digestive juice, and β-glucosidase from Aspergillus was obtained from Amano Pharmaceutical Co. The sample was dissolved in H_2O or dil. EtOH and enzyme powder added. The mixture was shaken at 30–38° for 1–2 days. Saponification: The acetate was dissolved in MeOH and a same amount of $KHCO_3$ in H_2O added. The vol. of H_2O was adjusted to 25% of the total. The soln was allowed to stand at room temp for 1–2 weeks. Acetolysis: The sample was heated at 100° with Ac_2O and a small amount of $ZnCl_2$ for 30 min, poured into ice-H_2O and the ppt filtered or extracted with solvent.

Extraction and fractionation. Fresh leaves of *N. odorum* were air-dried at 15–20° and powdered. The powdered leaves (2 kg) were percolated with MeOH, and extract concentrated, and diluted with an equal vol. of H_2O. The ppt was filtered and the filtrate extracted with C_6H_6 (Fraction 1) (5 g), $CHCl_3$ (Fraction 2) (26·9 g) and $CHCl_3$–EtOH (2:1) (Fraction 3) (37·6 g), successively.

Oleandrigenin β-D-glucosyl-β-D-diginoside (**1A**). Fraction 2 was subjected to successive column chromatography using Solvent 2 and Solvent 3 and the fractions showing the main spot of Fraction 2 were combined. On crystallization (EtOAc–EtOH) needles were obtained (**1A**) (0·011%), m.p. 178–181°, $[\alpha]_D$ −34·0° (*c* 0·5) ([M]$_D$ −251°), UV λ_{max} 222 nm (ε 15500), (Found: C, 60·0; H, 8·0. $C_{38}H_{58}O_{14}$. H_2O requires: C, 60·3; H, 8·0%). **1A** *Acetate.* m.p. 117–120°, $[\alpha]_D$ −27·3° (*c* 0·2), (Found: C, 60·4; H, 7·3. $C_{46}H_{66}O_{18}$ requires: C, 60·9; H, 7·3%). Acid hydrolysis: **1A** (150 mg) was hydrolyzed with 20 ml of the reagent (Condition 2), and aglycone (m.p. 224–229°, $[\alpha]_D$ −6·4° (*c* 0·5)) was identified as oleandrigenin by direct comparison with authentic sample. Sugar: PC R_f 0·60, 0·06 (specimen L-oleandrose 0·72, D-diginose 0·60, D-glucose 0·06, Solv. 1). **1A** was refluxed with 1 N H_2SO_4 for 1 hr and subjected to quantitative estimation of glucose using anthrone; glucose: 1·2 mol. Enzymatic hydrolysis: **1A** (200 mg) was treated with snail esterase and the product crystallized from hexane–EtOAc to give 55 mg of needles, m.p. 219–221°, $[\alpha]_D$ +3·5° (*c* 0·1), (Found: C, 67·2; H, 8·7. Calcd. for $C_{30}H_{46}O_8$: C, 67·4; H, 8·7%). On admixture with authentic desacetylnerigoside (m.p. 215–220°, $[\alpha]_D$ +5·0°), no m.p. depression was observed. Saponification: A product from 300 mg of **1A** was purified through column (Solvent 1), followed by crystallization from MeOH to give 40 mg of needles, m.p. 211–216°, $[\alpha]_D$ −6·6° (*c* 0·1), UV λ 220 nm (ε 14600).

Oleandrigenin β-D-glucoside (**1b**). The mother liquor of **1a** was chromatographed successively using Solv. 2 and Solv. 3 to give **1b** as homogeneous powder (0·003%). **1b** *Acetate.* m.p. 113–117°, $[\alpha]_D$ −26·7° (*c* 0·7) ([M]$_D$ −139°), UV λ 217 nm (ε 16900), MS: *m/e* 684·315, *m/e* 331·104. $C_{37}H_{48}O_{12}$ (M^+ − H_2O–HOAc) requires: 684·315, *m/e* 331·104. $C_{14}H_{19}O_9$ (glucose pentaacetate–$MeCO_2$) requires: 331·103. Acid hydrolysis of desacetyl **1b**; Desacetyl **1b** was treated under Mannich's conditions. The aglycone was indentical with authentic gitoxigenin on TLC. Sugar: PC R_f 0·05 (D-glucose 0·05, Solv. 1), R_f 0·21 (D-glucose 0·21, Solv. 2). Enzymatic hydrolysis: **1b** (140 mg) was treated with snail enzyme. The hydrolyzate was extracted with $CHCl_3$, purified through a column (Solv. 4) and crystallized from hexane EtOAc to give 10 mg of prisms, m.p. 226–233°, UV λ 217 nm (ε 13200). No m.p. depression was observed on admixture with authentic oleandrigenin, and the IR of both were superimposable.

Oleadrigenin β-D-glucosyl-β-D-digitaloside (16-O-*acetyl digitalinum verum* (**1c**). The latter effluent on column chromatography of **1a** was again passed through column and fractionated using Solv. 2 to afford an amorphous solid (**1c**) (0·005%). **1c** *Acetate.* m.p. 220–225°, $[\alpha]_D$ −22·2° (*c* 0·2). **1c** was saponified and purified through column. On crystallization from MeOH–H_2O, prisms were obtained, m.p. 235–238°, UV λ_{max} 217 nm (ε 19200), and in good agreement with authentic digitalinum verum on direct comparison (m.m.p., IR, TLC).

Δ^{16}-Dehydroadynerigenin D-glucosyl-β-D-digitaloside (**3c**). The fraction following **1b** on column chromatography with Solv. 3 was acetylated and the acetate was obtained as needles (under 0·001%) on crystallization from EtOH, m.p. 221–225°, $[\alpha]_D$ +20·0° (*c* 0·4), UV λ_{max} 267 nm (ε 10800), (Found: C, 60·3; H, 6·8. $C_{46}H_{63}O_{19}$ requires: C, 60·1; H, 6·9%). Acid hydrolysis: Acetate of **3c** was saponified and **3c** obtained was hydrolyzed under Mannich's conditions. The aglycone was identified on TLC as Δ^{16}-dehydroadynerigenin, accompanying Δ^{16}-dehydroadynerigenin β-D-digitaloside [7]. From the H_2O layer, glucose and digitalose was detected. PC R_fs: 0·36, 0·05, Solv. 1 and 0·52, 0·22, Solv. 2.

Oleandrigenin β-gentiobiosyl-α-L-oleandroside (gentiobiosyl oleandrin) (**1d**). Fraction 3 was subjected to successive column chromatography with Solv. 2 and then with Solv. 3. Crystallization from MeOH gave needles (**1d**) (0·06%), soluble in water, m.p. 169–173°, $[\alpha]_D$ −47·8° (*c* 0·5), ([M]$_D$ −431°), UV λ_{max} 214 nm (ε 15600). (Found: C, 57·2; H, 7·6. $C_{44}H_{68}O_{19}$. H_2O requires C, 57·5; H, 7·6%). (No. 3 of Görlich: m.p. 180–200°, $[\alpha]_D$ +8·3°) [5]. **1d** *Acetate.* m.p. 130–133°, $[\alpha]_D$ −42·8 (*c* 0·5). Acid hydrolysis: **1d** (200 mg) was hydrolyzed under Condition

* In the study of the oven-dried leaves [6], nerigoside was detected on TLC but not isolated in crystalline form.

2 to give 30 mg of aglycone (MeOH–Et_2O), m.p. 225–232°, $[\alpha]_D$ −6·4° (*c* 0·1), and was in good agreement with authentic oleandrigenin on direct comparison (m.m.p., IR, TLC). Sugar: PC R_f 0·75, 0·11, 0·03 (L-oleadnrose 0·75, D-diginose 0·72, D-glucose 0·11, gentiobiose 0·03, Solv. 1). R_f 0·76, 0·20, 0·07 (L-oleandrose 0·76, D-diginose 0·74, D-glucose 0·20, gentiobiose 0·07, Solv. 2). Quantitative estimation of glucose was conducted as above and gave 2·0 mol. Enzymatic hydrolysis: **1d** (300 mg) was treated with β-glucosidase and the product extracted with $CHCl_3$–EtOH (2:1). The extract was purified through column (Solv. 2), followed by crystallization from MeOH–H_2O to give 80 mg of needles (**1e**), m.p. 158–161°, $[\alpha]_D$ −54·4° (*c* 0·5) ($[M]_D$ −402°). UV λ_{max} 214 nm (ε 18100). Quantitative determination of glucose: 1·04 mol. (Found: C, 61·6; H, 7·9. Calcd. for $C_{38}H_{54}O_{14}$ (monoglucosyl oleandrin): C, 61·8; H, 7·9%). Acetolysis: **1d** acetate was acetolyzed and 10 mg of needles were obtained on crystallization from EtOH, m.p. 192–193°, $[\alpha]_D$ +48·7° (*c* 0·4). On direct comparison with authentic sample (m.p. 189–190°, $[\alpha]_D$ +50·0°), it was identified as α-gentiobiose octaacetate.

Digitoxigenin β-gentiobiosyl-β-D-diginoside (gentiobiosyl odoroside A) (**2a**). The fractions following **1d** on column chromatography were combined and acetylated. The acetate was successively purified through column using Solv. 4. On crystallization from EtOH, **2a** acetate was obtained as needles (0·005%), m.p. 167–169°, $[\alpha]_D$ −24·5° (*c* 0·6), UV λ 215 nm (ε 19300). (Found: C, 58·4; H, 7·0. $C_{56}H_{80}O_{24}.H_2O$ requires: C, 58·2; H, 7·2%). On saponification of **2a** acetate, amorphous **2a** was obtained, $[\alpha]_D$ −28·1° (*c* 1·9), UV λ_{max} 217 nm (ε 12500). Acid hydrolysis: **2a** (50 mg) was hydrolyzed under Condition 2. Digitoxigenin and anhydrodigitoxigenin were detected on TLC. Sugar portion was again refluxed with 1 N H_2SO_4 for 1 hr and subjected to PC, R_f 0·65, 0·13 (L-oleandrose 0·69, D-diginose 0·66, D-digitalose 0·41, D-glucose 0·13, Solv. 1). Enzymatic hydrolysis: **2a** was treated with β-glucosidase and the product was crystallized from MeOH to give needles, m.p. 240–245°, $[\alpha]_D$ −20·0° (*c* 0·8), UV λ 217 nm (ε 13600), which was further hydrolyzed to digitoxigenin (detected by TLC) and diginose and glucose under Condition 2. Acetolysis: **2a** acetate (200 mg) was acetolyzed. A product was purified through column with Solv. 4 and crystallized from EtOH to give 20 mg of α-gentiobiose octaacetate, m.p. 192–193°, $[\alpha]_D$ +48·7° (*c* 0·4).

Odoroside G (**2b**). The most polar fraction on chromatography of Fraction 3 with Solvent 3 was further fractionated with Solvent 2, successively, and the last fraction of the final column was acetylated. The acetate was purified through column, followed by crystallization from EtOH to give needles (**2b** acetate), m.p. 236–238°, $[\alpha]_D$ −22·3° (*c* 0·9), UV λ_{max} 217 nm (ε 13800). No m.p. depression was observed on admixture with authentic odoroside G acetate (m.p. 232–235°, $[\alpha]_D$ −20·0°).

Survey for gentiobiosyl nerigoside. Fraction 3 (6·9 g) was treated with β-glucosidase, and the resulting mixture was extracted with $CHCl_3$. The extract was subjected to column chromatography with Solv. 1 and then with Solv. 2. The fractions were examined by TLC. The following glycosides were found: monoglucosides of oleandrin (major), odoroside A, adynerin, Δ^{16}-dehydroadynerin, adynerigenin digitaloside, and of Δ^{16}-dehydroadynerigenin digitaloside, and one unknown spot. Monoglucosyl nerigoside and nerigoside were not detected.

Adynerigenin- and Δ^{16}-dehydroadynerigenin-gentiobiosyl-β-D-diginoside. The fractions immediately following **1d** were successively chromatographed with Solv. 2 and the amorphous substance (A), showing homogeneous yellow spot with $SbCl_3$ on TLC, was obtained (*ca* 0·03%). Acid hydrolysis: A (70 mg) was refluxed under Condition 1. $CHCl_3$ extract of the hydrolyzate was subjected to column chromatography with Solv. 4. Fraction 1 was crystallized from MeOH to give 18 mg of prisms, m.p. 178–181°. On admixture with $\Delta^{5,9,14}$-anhydroadynerigenin (m.p. 176–179°), no m.p. depression was observed and IR of the both samples were in good agreement. Fraction 2 was crystallized from MeOH to give 3 mg of prisms, m.p. 187–190°. No m.p. depression was observed on admixture with Δ^{16}-dehydroadynerigenin (m.p. 182–184°). Enzymatic hydrolysis: A (70 mg) was treated with snail esterase. The $CHCl_3$ extract was purified through column with Solv. 4, followed by crystallization from MeOH to give 2 mg of prisms, m.p. 218–222°. On mixed fusion with adynerin, no m.p. depression was observed. In mother liquor portion, Δ^{16}-dehydroadynerin was detected by TLC. Acetolysis: A-Acetate (45 mg) was treated and a product was purified through column, followed by crystallization from EtOH to give α-gentiobiose octaacetate (m.p. 190–194°).

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