2′-*O*-Acetates of Obeside B, Honghelin, and Obebiosides A and B from *Adenium obesum*. (Studies on the Constituents of *Adenium*. II)[1]

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2′-*O*-Acetyl-β-D-thevetosides and β-D-glucosyl(1→4)-2′-*O*-acetyl-β-D-thevetosides of oleandrigenin and digitoxigenin were isolated along with D-cymarosides, D-thevetosides and D-digitalosides from the roots and the stems of *Adenium obesum* ROEM. *et* SCHULT. 4-*O*-β-D-Glucopyranosyl-D-cymaritol was isolated from the polar fraction.

Keywords *Adenium obesum*; Apocynaceae; cardiac glycoside; 2′-*O*-acetyl cardiac glycoside; 2′-*O*-acetylobebioside B; oleandrigenin β-D-glucosyl(1→4)-2′-*O*-acetyl-β-D-thevetoside; 4-*O*-β-D-glucosyl-D-cymaritol

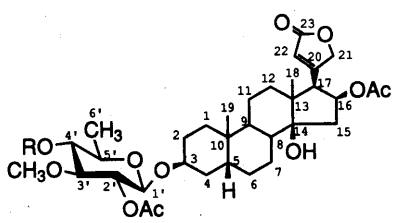
In the preceding paper,[1] we described the isolation of thirty cardiac glycosides from the roots and the stems of *Adenium obesum* ROEM. *et* SCHULT., including fifteen known glycosides, mostly D-cymarosides and D-digitalosides of digitoxigenin and oleandrigenin,[2] along with neridienone A and 16,17-dihydroneridienone A.[3] The structures of eleven glycosides having new combinations of oleandrigenin and digitoxigenin with D-thevetose, glucosyl- and gentiobiosyl-D-thevetose were identified, while four minor glycosides (**27**—**30**) remained undetermined.[1] This paper deals with **27**—**30** having an acetyl residue at 2-OH of the D-thevetose moiety, and 4-*O*-β-D-glucosyl-D-cymaritol (**33**).

When the cardiac glycosides were isolated from the roots and the stems, two glycosides (**27**, **28**) showing intermediate polarity on chromatography between the cymarosides and the thevetosides, and two (**29**, **30**) showing intermediate polarity between the glucosylcymarosides and the glucosylthevetosides were obtained.

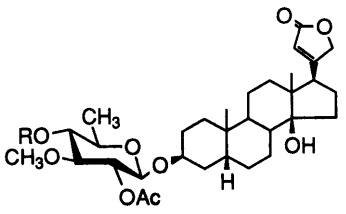
In the fast atom bombardment (FAB) mass spectrum (MS) of **27**, an $[M+Na]^+$ peak was observed at *m/z* 657, suggesting the molecular formula to be $C_{34}H_{50}O_{11}$, 42 mass unit larger than obeside B (oleandrigenin β-D-thevetoside).[1] In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, a 3H singlet signal at δ 2.17 suggested the presence of one additional acetyl group. Since the proton signals due to the 16-*O*-actyl group and H-16 as well as H-3, H-17, H-18, H-19, H-21 and H-22 were observed with the usual chemical shifts for oleandrigenin, the acetyl group seemed to be linked to the sugar moiety. A methoxyl proton signal at C-3′ of the sugar moiety was observed at the higher field (δ 3.67) in comparison with that of obeside B (δ 3.89). Each proton signal in the sugar moiety was assigned on the basis of the cross peaks in ^{1}H–^{1}H correlation spectroscopy (COSY), and the coupling constants of H-1′—H-4′ were observed as 8 Hz (doublet, H-1′; triplets, H-2′,3′,4′) as in obeside B. Since H-2′ was observed at lower field (δ 5.38), the acetyl group was suggested to be linked to 2-OH of D-thevetose. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, C-1′ and C-3′ were observed at higher field, at δ 99.5 and 85.4, respectively, in comparison with those of obeside B (δ 102.9 and 88.1). On acetylation of **27**, the resultant acetate was confirmed to be obeside B-acetate by direct comparison. Compound **27** was thus determined to be oleandrigenin 2′-*O*-acetyl-β-D-thevetoside (2′-*O*-acetylobeside B).

In the ^{1}H-NMR spectrum, **28** showed the same signals due to the 2′-*O*-acetyl-β-D-thevetosyl moiety as those of **27**, and the aglycone was assignable to digitoxigenin from the signals due to H-3, H-17, H-18, H-19, H-21a, b and H-22. Based on the $[M+Na]^+$ peak at *m/z* 599.3203 ($C_{32}H_{48}NaO_9$) as well as the ^{1}H-NMR signals, **28** was assigned as digitoxigenin 2′-*O*-acetyl-β-D-thevetoside (2′-*O*-acetylhonghelin).

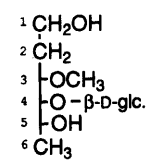
In the ^{1}H-NMR spectra of **29** and **30**, the presence of one acetyl signal at δ 2.13 (**29**) or 2.10 (**30**) in addition to the 16-*O*-acetyl residue in **29**, was observed along with one each of a methoxyl group, a *sec*-methyl group and a pair of primary carbinyl protons assignable to H-6a, b of a hexopyranose. Since two anomeric protons were observed as doublets (δ 4.72, 5.06; each $J=8$ Hz, **29**; δ 4.71, 5.05; each $J=8$ Hz, **30**) and H-2′ was observed at lower field (δ 5.36) as in **27** and **28**, the sugar moieties seemed to be composed of 2-*O*-acetyl-D-thevetose and D-glucose. The FAB-MS of **29** afforded an $[M+Na]^+$ peak at *m/z* 819, while **30** showed an $[M-1]^-$ peak at *m/z* 737, along with peaks at *m/z* 575, 533 and 373 [aglycone−1]$^-$ in the negative FAB-MS. The peaks in the FAB-MS were consistent with the ^{1}H-NMR considerations. Based on the glycosylation shift of C-4 of D-thevetose (+7 ppm) in the ^{13}C-NMR spectrum, the linkage



27: R = H
29: R = β-D-glucose



28: R = H
30: R = β-D-glucose



33

Chart 1

of glucose was determined to be at 4-OH of the thevetose moiety. Compounds **29** and **30** were therefore determined to be β-D-glucosyl(1→4)-2′-*O*-acetyl-β-D-thevetosides of oleandrigenin and digitoxigenin (2′-*O*-acetylobebiosides B and A), respectively.

When the MeOH percolate of the plant materials was partitioned with BuOH/water, and the water layer was further chromatographed on an MCI-gel column, and then on a YMC-gel column, **33** was isolated as a solid. In the FAB-MS, **33** afforded an $[M+Na]^+$ peak at *m/z* 349.1451, indicating the molecular formula to be $C_{13}H_{26}O_9$. Since the signals due to a *sec*-methyl group and a methoxyl group were observed in the ^{1}H-NMR spectrum and the presence of one glucose unit, a primary carbinol (δ 58.4) and a methylene group (δ 33.1) was suggested by the ^{13}C-NMR spectrum, **33** was considered to be glucosyl-cymaritol. Finally, the structure was confirmed by direct comparison with authentic 4-*O*-β-D-glucosyl-D-cymaritol which was prepared by $NaBH_4$ reduction of strophanthobiose.

It should be noted that 2′-*O*-acetyl-β-D-thevetosides and glucosyl-2′-*O*-acetylthevetosides of digitoxigenin and oleandrigenin, but not 2′-*O*-acetyl-β-D-digitalosides, were obtained from this plant. The seed kernels of *Cerbera* spp. contain digitoxigenin 2′-*O*-acetyl-L-thevetoside as well as its glucosyl and gentiobiosyl homologues, along with acetyl free glycosides.[4]

Experimental

Melting points, optical rotations, ^{1}H-NMR, ^{13}C-NMR and FAB-MS were obtained as described in the preceding paper,[1] and the samples for NMR measurements were dissolved in pyridine-d_5. Column chromatography and thin-layer chromatography (TLC) were conducted with the following solvent systems: solvent 1, benzene–acetone (7:1—5:1); solvent 2, hexane–EtOAc (2:1—1:2); solvent 3, $CHCl_3$–MeOH–H_2O (bottom layer); solvent 4, EtOAc–MeOH–H_2O (upper layer).

Isolations of compounds **27**—**30** from the fresh roots (4.5 kg) and the stems (3.0 kg) of *Adenium obesum* ROEM. *et* SCHULT. were described in the preceding paper.[1]

Oleandrigenin 2′-*O*-Acetyl-β-D-thevetoside (2′-*O*-Acetylobeside B, 27) Solid, (50 mg from the roots), $[\alpha]_D^{26}$ −21.8° (*c*=0.28, MeOH), *Anal.* Calcd for $C_{34}H_{50}O_{11}\cdot 1.5H_2O$: C, 61.71; H, 8.07. Found: C, 61.86; H, 7.98. FAB-MS *m/z*: 657 $[M+Na]^+$. ^{1}H-NMR δ: 0.93, 1.08 (3H each, s, H-18,19), 1.54 (3H, d, *J*=6 Hz, H-6′), 1.86 (3H, s, 16-OAc), 2.06 (1H, dd, *J*=15, 2 Hz, H-15a), 2.17 (3H, s, 2′-OAc), 2.81 (1H, dd, *J*=15, 9 Hz, H-15b), 3.38 (1H, d, *J*=9 Hz, H-17), 3.61, 3.62 (1H each, t, *J*=8 Hz, H-3′,4′), 3.67 (3H, s, 3′-OMe), 3.68 (1H, m, H-5′), 4.25 (1H, br s, H-3), 4.79 (1H, d, *J*=8 Hz, H-1′), 5.23 (1H, dd, *J*=18, 1 Hz, H-21a), 5.38 (1H, t, *J*=8 Hz, H-2′), 5.41 (1H, dd, *J*=18, 2 Hz, H-21b), 5.64 (1H, s, –OH), 5.68 (1H, dt, *J*=2, 9 Hz, H-16), 6.33 (1H, br s, H-22). ^{13}C-NMR δ: 16.3 (C-18), 18.2 (C-6′), 20.6 (16-OCO$\underline{C}$H$_3$), 21.0 (2′-OCO$\underline{C}$H$_3$), 21.1, 21.7 (C-11, 7), 24.0 (C-19), 27.0, 27.1 (C-2, 6), 30.1, 30.5 (C-1, 4), 35.4 (C-10), 35.8, 36.8 (C-5, 9), 38.9 (C-12), 41.2 (C-15), 42.0 (C-8), 50.4 (C-13), 56.7 (C-17), 59.9 (3′-OMe), 72.8 (C-5′), 73.7 (C-3), 74.2 (C-2′), 74.9 (C-16), 75.7 (C-4′), 76.2 (C-21), 83.4 (C-14), 85.1 (C-3′), 99.3 (C-1′), 121.6 (C-22), 169.5, 169.6 (–O$\underline{C}$OCH$_3$), 170.1 (C-20), 174.0 (C-23).

Upon acetylation of **27** (5 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature for 24 h, **27**-acetate was obtained as needles (mp 231—234 °C, FAB-MS *m/z*: 699 $[M+Na]^+$), and identified as obeside B acetate by mixture melting point determination and by TLC comparison (solvents 1 and 2) with an authentic sample.

Digitoxigenin 2′-*O*-Acetyl-β-thevetoside (2′-*O*-Acetylhonghelin, 28) Prisms from EtOH–H_2O, mp 209—211 °C, (5 mg from the roots) $[\alpha]_D^{23}$ −15.1° (*c*=0.45, MeOH). FAB-MS *m/z*: 599.3203 (Calcd for $C_{32}H_{48}NaO_9$ 599.3197). ^{1}H-NMR δ: 0.94, 1.02 (3H each, s, H-18,19), 1.55 (3H, d, *J*=6 Hz, H-6′), 2.17 (3H, s, 2′-OAc), 2.80 (1H, dd, *J*=8, 5 Hz, H-17), 3.628, 3.634 (1H each, t, *J*=8 Hz, H-3′, 4′), 3.67 (3H, s, 3′-OMe), 3.68 (1H, m, H-5′), 4.26 (1H, br s, H-3), 4.79 (1H, d, *J*=8 Hz, H-1′), 5.03 (1H, dd, *J*=18, 1 Hz, H-21a), 5.20 (1H, s, –OH), 5.30 (1H, br d, *J*=18 Hz, H-21b), 5.33 (1H, t, *J*=8 Hz, H-2′), 6.13 (1H, br s, H-22).

Oleandrigenin β-D-Glucosyl-2′-*O*-acetyl-β-D-thevetoside (2′-*O*-Acetylobebioside B, 29) Solid (240 mg from the roots; 65 mg from the stems), $[\alpha]_D^{24}$ −19.7° (*c*=1.50, MeOH), *Anal.* Calcd for $C_{40}H_{60}O_{16}\cdot 2H_2O$: C, 57.68; H, 7.75. Found: C, 57.40; H, 7.72. FAB-MS *m/z*: 819 $[M+Na]^+$. ^{1}H-NMR δ: 0.92, 1.08 (3H each, s, H-18, 19), 1.72 (3H, d, *J*=6 Hz, H-6′), 1.86 (3H, s, 16-OAc), 2.06 (1H, dd, *J*=15, 2 Hz, H-15a), 2.13 (3H, s, 2′-OAc), 2.81 (1H, dd, *J*=15, 10 Hz, H-15b), 3.38 (1H, d, *J*=9 Hz, H-17), 3.73 (1H, t, *J*=9 Hz, H-3′), 3.75 (3H, s, 3′-OMe), 3.75 (1H, m, H-5′), 3.86 (1H, t, *J*=9 Hz, H-4′), 3.92 (1H, m, H-5″), 3.99 (1H, t, *J*=8 Hz, H-2″), 4.15—4.22 (3H, H-3″, 4″, 3), 4.33 (1H, dd, *J*=11, 5 Hz, H-6″a), 4.49 (1H, dd, *J*=11, 2 Hz, H-6″b), 4.72 (1H, d, *J*=8 Hz, H-1′), 5.06 (1H, d, *J*=8 Hz, H-1″), 5.23, 5.41 (1H, each, dd, *J*=18, 1 Hz, H-21a, b), 5.36 (1H, dd, *J*=8, 9 Hz, H-2′), 5.64 (1H, s, –OH), 5.68 (1H, dt, *J*=2, 9 Hz, H-16), 6.33 (1H, br s, H-22). ^{13}C-NMR δ: 16.2 (C-18), 18.4 (C-6′), 20.6, 21.0 (–O$\underline{C}$O$\underline{C}$H$_3$), 21.1, 21.7 (C-7, 11), 23.9 (C-19), 26.9, 27.0 (C-2, 6), 30.1, 30.4 (C-1, 4), 35.3 (C-10), 35.7, 36.8 (C-5,9), 38.9 (C-12), 41.2 (C-15), 41.9 (C-8), 50.4 (C-13), 56.7 (C-17), 59.8 (3′-OMe), 63.0 (C-6″), 71.9, 72.1 (C-4″, 5′), 73.7, 73.9 (C-2′, 3), 74.9 (C-16), 75.7 (C-2″), 76.2 (C-21), 78.1, 78.6 (C-3″, 5″), 82.9, 83.3 (C-3′, 4′), 83.4 (C-14), 99.2 (C-1′), 104.8 (C-1″), 121.6 (C-22), 169.4, 169.6 (–$\underline{C}$OCH$_3$), 170.1 (C-20), 174.1 (C-23).

Digitoxigenin β-D-Glucosyl(1→4)-2′-*O*-acetyl-β-D-thevetoside (2′-*O*-Acetylobebioside A, 30) Prisms from MeOH, mp 238—243 °C (86 mg from the roots; 25 mg from the stems), $[\alpha]_D^{26}$ −9.5° (*c*=2.10, MeOH), *Anal.* Calcd for $C_{38}H_{58}O_{14}\cdot 1.5H_2O$: C, 59.59; H, 8.03. Found: C, 59.77; H, 8.29. Negative FAB-MS *m/z*: 737 $[M-1]^-$, 575 $([M-1]^- - glc)$, 533 $([M-1]^- - glc-Ac)$, 373 [aglycone − 1]$^-$. ^{1}H-NMR δ: 0.92, 1.02 (3H, each, s, H-18, 19), 1.72 (3H, d, *J*=6 Hz, H-6′), 2.10 (3H, s, 2′-OAc), 2.79 (1H, dd, *J*=9, 5 Hz, H-17), 3.73 (1H, t, *J*=8 Hz, H-3′), 3.75 (3H, s, 3′-OMe), 3.75 (3.75 (1H, m, H-5′), 3.86 (1H, t, *J*=9 Hz, H-4′), 3.92 (1H, m, H-5″), 3.98 (1H, t, *J*=8 Hz, H-2″), 4.14—4.21 (3H, H-3″, 4″, 3), 4.32 (1H, dd, *J*=12, 5 Hz, H-6″a), 4.48 (1H, dd, *J*=12, 2 Hz, H-6″b), 4.71 (1H, d, *J*=8 Hz, H-1′), 5.02, 5.29 (1H each, br d, *J*=17 Hz, H-21a, b), 5.05 (1H, d, *J*=7 Hz, H-1″), 5.36 (1H, t, *J*=8 Hz, H-2′), 6.13 (1H, br s, H-22). ^{13}C-NMR δ: 16.2 (C-18), 18.4 (C-6′), 21.0 (–OCO$\underline{C}$H$_3$), 21.5, 22.0 (C-7, 11), 24.0 (C-19), 26.9, 27.2, 27.3 (C-2, 6, 16), 30.1, 30.5 (C-1, 4), 33.1 (C-15), 35.5 (C-10), 35.9, 36.9 (C-5, 9), 39.8 (C-12), 41.9 (C-8), 50.1 (C-13), 51.4 (C-17), 59.8 (3′-OMe), 63.0 (C-6″), 71.9, 72.0 (C-4″, 5′), 73.7 (C-21), 73.9, 74.0 (C-2′, 3), 75.7 (C-2″), 78.0, 78.6 (C-3″, 5″), 82.9, 83.3 (C-3′, 4′), 84.7 (C-14), 99.1 (C-1′), 104.8 (C-1″), 117.7 (C-22), 169.4 (–O$\underline{C}$OCH$_3$), 174.4 (C-23), 175.9 (C-20).

Isolation of 4-*O*-β-D-Glucosyl-D-cymaritol (33) The H_2O layer, after the MeOH percolates of the roots (4.5 kg) and stems (3.0 kg) had been partitioned with BuOH,[1] was passed through an MCI-gel column (Mitsubishi Chem. Co., HP-20) and the column was eluted with H_2O and 25% MeOH. The eluate with 25% MeOH (4.4 g) was then chromatographed on a YMC-gel column (Yamamura Chem. Co.) and the 7% CH_3CN eluate was purified on a silica gel column with solvent 3 (7:2:0.8—7:3:1.2) to afford **33** (38 mg) as a solid. $[\alpha]_D^{23}$ −18.5° (*c*=2.25, MeOH). FAB-MS *m/z*: 349.1451. Calcd for $C_{13}H_{26}NaO_9$: 349.1474. ^{1}H-NMR δ: 1.57 (3H, d, *J*=6 Hz, H-6), 2.47, 2.57 (1H each, m, H-2a, b), 3.38 (3H, s, 3-OMe), 3.80 (1H, m, H-3), 3.92 (1H, m, H-5′), 4.01 (1H, t, *J*=8 Hz, H-4′), 4.05 (1H, t, *J*=8 Hz, H-2′), 4.09—4.22 (2H, m, H-1), 4.17 (1H, t, *J*=8 Hz, H-3′), 4.21 (1H, dd, *J*=12, 6 Hz, H-6′a), 4.36 (1H, dd, *J*=8, 4 Hz, H-4), 4.46 (1H, m, H-5), 4.54 (1H, dd, *J*=12, 2 Hz, H-6′b), 5.19 (1H, d, *J*=8 Hz, H-1′). ^{13}C-NMR δ: 18.3 (C-6), 33.1 (C-2), 57.0 (3-OMe), 58.4 (C-1), 63.3 (C-6′), 68.0 (C-5), 72.0 (C-4′), 76.0 (C-2′), 78.6 (C-5′), 78.7 (C-3′), 79.4 (C-3), 86.3 (C-4), 106.9 (C-1′).

Strophanthobiose (50 mg), prepared from k-strophanthin-β (150 mg) by refluxing with 0.05 N H_2SO_4–50% dioxane (3 ml) for 1 h, was dissolved in MeOH–EtOH (1:1) (2 ml) and stirred with 50 mg of $NaBH_4$ for 2 h at room temperature. The reaction mixture was diluted with H_2O and extracted with BuOH. The BuOH extract was chromatographed on a silica gel column with solvent 3 (7:3:1.6) to give a homogeneous solid (36 mg). $[\alpha]_D^{27}$ −15.7° (*c*=1.80, MeOH). In a parallel run with **33**, the same *Rf* value was observed on TLC (solvent 3). The ^{1}H-NMR spectra of the two compounds were superimposable, as were the ^{13}C-NMR spectra.

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