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 $\rightarrow$ 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-acetyl-obebioside B; oleandrigenin β -D-glucosyl(1 $\rightarrow$ 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-dihydroneidienone 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 glucosyl-thevetosides 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 obese B (oleandrigenin β -D-thevetoside).¹⁾ In the proton nuclear magnetic resonance (1H -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-acetyl 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 obese B (δ 3.89). Each proton signal in the sugar moiety was assigned on the basis of the cross peaks in 1H - 1H 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 obese 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 obese B (δ 102.9 and 88.1). On acetylation of 27, the resultant acetate was confirmed to be obese B-acetate by direct comparison. Compound 27 was thus determined to be oleandrigenin 2'-O-acetyl- β -D-thevetoside (2'-O-acetyl-obeside B).

In the 1H -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 1H -NMR signals, 28 was assigned as digitoxigenin 2'-O-acetyl- β -D-thevetoside (2'-O-acetyl-honghelin).

In the 1H -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 carbonyl 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 1H -NMR considerations. Based on the glycosylation shift of C-4 of D-thevetose (+7 ppm) in the ^{13}C -NMR spectrum, the linkage

Chart 1

of glucose was determined to be at 4-OH of the thevetoside moiety. Compounds **29** and **30** were therefore determined to be β -D-glucosyl(1 $\rightarrow$ 4)-2'-O-acetyl- β -D-thevetosides of oleandrigenin and digitoxigenin (2'-O-acetyllobebiosides 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 1H -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, 1H -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-Acetyllobeside B, 27) Solid, (50 mg from the roots), $[\alpha]_D^{25} -21.8^\circ$ ($c=0.28$, 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 $[M+Na]^+$. 1H -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, brs, 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, brs, H-22). ^{13}C -NMR δ : 16.3 (C-18), 18.2 (C-6'), 20.6 (16-OCOCH₃), 21.0 (2'-OCOCH₃), 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 (-OCOCH₃), 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 obese 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-Acetylhomghelin, 28) Prisms from EtOH- H_2O , mp 209–211°C, (5 mg from the roots) $[\alpha]_D^{23} -15.1^\circ$ ($c=0.45$, MeOH). FAB-MS m/z : 599.3203 (Calcd for $C_{32}H_{48}NaO_9$, 599.3197). 1H -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, brs, 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, brs, H-22).

Oleandrigenin β -D-Glucosyl-2'-O-acetyl- β -D-thevetoside (2'-O-Acetyllobeside B, 29) Solid (240 mg from the roots; 65 mg from the stems), $[\alpha]_D^{24} -19.7^\circ$ ($c=1.50$, 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 $[M+Na]^+$. 1H -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, brs, H-22). ^{13}C -NMR δ : 16.2 (C-18), 18.4 (C-6'), 20.6, 21.0 (-OCOCH₃), 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 (-OCOCH₃), 170.1 (C-20), 174.1 (C-23).

Digitoxigenin β -D-Glucosyl(1 $\rightarrow$ 4)-2'-O-acetyl- β -D-thevetoside (2'-O-Acetyllobeside A, 30) Prisms from MeOH, mp 238–243°C (86 mg from the roots; 25 mg from the stems), $[\alpha]_D^{26} -9.5^\circ$ ($c=2.10$, 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 $[aglycone-1]^-$. 1H -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, brs, H-22). ^{13}C -NMR δ : 16.2 (C-18), 18.4 (C-6'), 21.0 (-OCOCH₃), 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 (-OCOCH₃), 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. $[\alpha]_D^{25} -18.5^\circ$ ($c=2.25$, MeOH). FAB-MS m/z : 349.1451. Calcd for $C_{13}H_{26}NaO_9$: 349.1474. 1H -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 0.05N 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). $[\alpha]_D^{27} -15.7^\circ$ ($c=1.80$, MeOH). In a parallel run with **33**, the same *Rf* value was observed on TLC (solvent 3). The 1H -NMR spectra of the two compounds were superimposable, as were the ^{13}C -NMR spectra.

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