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Studies on *Cerbera*. V.[1] Minor Glycosides of 17α-Digitoxigenin from the Stems of Genus *Cerbera*

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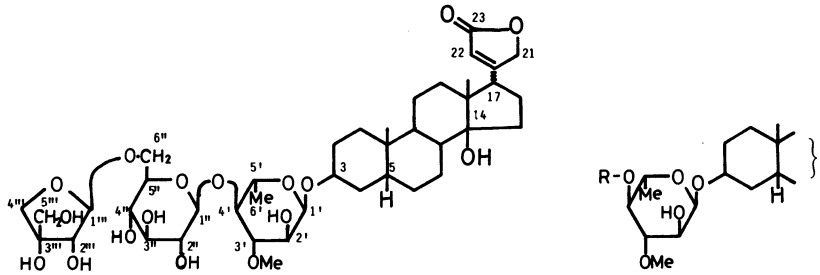
17α-Digitoxigenin apiosyl-glucosyl-thevetoside and cellobiosyl-thevetoside were isolated, together with known biosides and triosides, from the stems of *Cerbera manghas* L.

Keywords——*Cerbera manghas*; *Cerbera odollam*; Apocynaceae; cardenolide; cardenolide apioside; 17α-digitoxigenin cellobiosyl-thevetoside

In the preceding paper, we described the isolation of glucos-3-ulosyl-thevetosides of 17α-digitoxigenin and 17α-tanghinigenin, and of cerleaside A (oleagenin α-L-thevetoside) from the air-dried leaves of *Cerbera manghas* L. and *C. odollam* GAERTN.[2] Cerleaside B (oleagenin β-D-glucosyl-(1→4)-α-L-thevetoside) was obtained from the fresh leaves of *C. odollam*, and a gentiotriosyl-thevetoside of digitoxigenin was isolated from the fresh leaves of *C. manghas*.[1] Since the oleagenin glycosides, cerleaside A[2] and cerleaside B,[1] formed part of the major glycosides in the leaves of *C. odollam*, we have examined the glycosides from the stems of the two species. This paper deals with the isolation of 17α-digitoxigenin glycosides composed of apiose, glucose and thevetose in the sugar moiety (**1**), cellobiosyl-thevetoside (**2**), and gentiobiosyl-thevetoside (**3**), along with other known biosides, glucosyl-thevetosides of digitoxigenin (**4**), 17α-digitoxigenin (**5**), tanghinigenin (**6**), 17α-tanghinigenin (**7**) and oleagenin (cerleaside B) (**8**), and triosides, gentiobiosyl-thevetoside of digitoxigenin (thevetin B) (**9**), tanghinigenin (**10**) and 2′-*O*-acetyl-thevetin B (**11**).[3]

Compound **1**, showing intermediate polarity between biosides and triosides on thin layer chromatography (TLC), was isolated as a solid from the stems of *C. manghas*. Since the negative fast atom bombardment (FAB)-mass spectrum (MS) afforded the $(M-1)^-$ peak at *m/z* 827 and $(M-C_5H_8O_4-1)^-$ peak at *m/z* 695, **1** was considered to be a trioside having a pentose as the terminal sugar. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, the signals due to **5** were assignable; there was a downfield shift of C-6 of the glucose to which the terminal sugar is attached. The carbon signals of the pentose moiety observed at δ 111.1 (d), 80.3 (s), 77.8 (d), 75.0 (t), and 65.8 (t) and characteristic proton signals at δ 4.16 (2H, s), 4.34 and 4.56 (1H each, d, $J=9$ Hz) in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum suggest the terminal pentose to be D-apiose.[4] After acid hydrolysis of **1** with 0.5 N H_2SO_4–50% dioxane, apiose, **5**, and **5**-anhydride were detected on TLC.

Compound **2** showed the same *Rf* value as **9** (thevetin B) on TLC. In the negative FAB-MS, the peaks of $(M-1)^-$, $(M-\text{hexose}-1)^-$, $(M-(2\times\text{hexose})-1)^-$, and $(\text{genin}-1)^-$ were observed at *m/z* 857, 695, 533 and 373, respectively, suggesting **2** to be a trioside composed of hexobiosyl-thevetose. The proton and carbon signals due to an aglycone moiety were assignable to 17α-digitoxigenin. Since **2** was hydrolyzed to **5** with β-glucosidase, and the signals due to the hexobiosyl moiety were identified as those of methyl β-cellobioside on the



1

2 : R = −Glc $\overset{4\beta}{\leftarrow}$ Glc

3 : R = −Glc $\overset{6\beta}{\leftarrow}$ Glc

Chart 1

basis of the ^{13}C-NMR spectra,[5] **2** was determined to be 17α-digitoxigenin β-cellobiosyl-(1→4)-α-L-thevetoside.

Compound **3** was considered to be the 17α-isomer of **9** based on the ^{1}H- and ^{13}C-NMR spectra. The structure was confirmed by enzymic hydrolysis, yielding **5**.

Since the isolation of apiin, many glycosides having an apiosyl moiety have been reported. The occurrence of apiose in a cardenolide glycoside, **1**, is reported here for the first time. Compound **1** is named cerapioside. As with the glycosides from the leaves, the oleagenin glycoside was included among the major glycosides in the stems of *C. odollam*.

Experimental

Melting points, optical rotations, ^{1}H-NMR, ^{13}C-NMR and MS data were obtained as described in the preceding paper.[1] Column chromatography and TLC were conducted with the following solvent systems: solv. 1, $CHCl_3$–MeOH–H_2O (7:3:1, bottom layer); solv. 2, EtOAc–MeOH–H_2O (4:1:0.5); solv. 3, CH_3CN–H_2O. Spots on the TLC plate were detected by spraying diluted H_2SO_4 and heating the plate. High-performance liquid chromatography (HPLC) was run on a Waters ALC 200 equipped with a Radial Pack C_{18} column.

Isolation of 1, 2 and 3 from the Stems of *C. manghas*.——Air-dried stems of *C. manghas* L., cultivated in the greenhouse of Fukuoka University and harvested in Oct. 1986 (6 kg) were powdered and percolated with MeOH. The MeOH percolate was treated in the same manner as described in the preceding paper.[1] The bioside fraction was subjected to silica gel column chromatography with solv. 1 and solv. 2, reversed-phase column chromatography with RQ-1 (Fuji gel, solv. 3) and also HPLC (solv. 3) to isolate **1** (17 mg), **2** (6 mg), **3** (250 mg), **4** (85 mg), **5** (220 mg), **9** (200 mg), and **11** (7 mg).

Air-dried stems of *C. odollam* GAERTN. collected in Singapore in Feb. 1987 (1.2 kg), were treated in the same manner as described in the preceding paper[1] and the following glycosides were obtained; **3** (8 mg), **4** (25 mg), **5** (20 mg), **6** (80 mg), **7** (20 mg), **8** (13 mg), **9** (40 mg), and **10** (8 mg).

17α-Digitoxigenin β-D-Apiosyl-(1→6)-β-D-glucosyl-(1→4)-α-L-thevetoside (1) and Acid Hydrolysis of 1——A solid, $[\alpha]_D^{26}$ −60.3° (c=0.85, MeOH). Negative FAB-MS m/z: 827 ($C_{41}H_{64}O_{17}-1$)$^-$, 695 (M − apiose − 1)$^-$, 533 (695 − Glc)$^-$, 373 (aglycone − 1)$^-$. ^{1}H-NMR δ (ppm) (pyridine-d_5): 0.85, 1.19 (3H each, s, H-18, H-19), 1.80 (3H, d, J=6 Hz, H-6′), 3.42 (1H, t, J=9 Hz, H-17), 3.94 (3H, s, 3′-OMe), 4.16 (2H, s, H-5‴), 4.34, 4.56 (1H each, d, J=9 Hz, H-4‴), 4.71 (1H, d, J=2 Hz, H-2‴), 4.82, 4.97 (1H each, dd, J=18, 1 Hz, H-21a, b), 5.16 (1H, d, J=4 Hz, H-1′), 5.31 (1H, d, J=8 Hz, H-1″), 5.77 (1H, d, J=2 Hz, H-1‴), 6.12 (1H, d, J=1 Hz, H-22). ^{13}C-NMR δ (ppm) (pyridine-d_5): 30.3 (C-1), 26.8, 27.1 (C-2, C-6), 73.7, 73.6 (C-3, C-2′), 31.0 (C-4), 36.8 (C-5), 21.5 (C-7), 41.7 (C-8), 36.0 (C-9), 35.4 (C-10), 20.6 (C-11), 31.6 (C-12), 49.4 (C-13), 85.2 (C-14), 31.0 (C-15), 24.9 (C-16), 48.9 (C-17), 18.6, 18.5 (C-18, C-6′), 23.8 (C-19), 172.7 (C-20), 74.1 (C-21), 116.6 (C-22), 174.1 (C-23), 98.5 (C-1′), 85.3 (C-3′), 81.9 (C-4′), 67.5 (C-5′), 60.9 (3′-OMe), 104.9 (C-1″), 75.6 (C-2″), 78.3 (C-3″), 71.9 (C-4″), 76.9 (C-5″), 68.9 (C-6″), 111.1 (C-1‴), 77.8 (C-2‴), 80.3 (C-3‴), 75.0 (C-4‴), 65.8 (C-5‴). Compound **1** (3 mg) was refluxed with 0.5 N H_2SO_4–50% dioxane for 1 h, and the mixture was deacidified with IRA-410, and diluted with H_2O. The mixture was then extracted with BuOH. The BuOH extract was concentrated *in vacuo* and examined by TLC (solv. 1, *Rf* 0.65, 0.70) (**5**, 0.65; **5**-anhydride, 0.70). The H_2O layer was again evaporated to dryness *in vacuo* and the residue was examined by TLC (solv. 1, *Rf* 0.35, D-apiose: 0.35; solv. 2, *Rf* 0.45, D-apiose: 0.45).

17α-Digitoxigenin β-Cellobiosyl-(1→4)-α-L-thevetoside (2) and Enzymic Hydrolysis of 2——A solid, $[\alpha]_D^{24}$ −36.6° (c=0.25, MeOH). Negative FAB-MS m/z: 857 ($C_{42}H_{66}O_{18}-1)^-$, 695 (857-Glc)$^-$, 533 (695-Glc)$^-$, 373 (aglycone−1)$^-$. ^{1}H-NMR δ (ppm) (pyridine-d_5): 0.85, 1.19 (3H each, s, H-18, H-19), 1.63 (3H, d, J=6 Hz, H-6′), 3.42 (1H, t, J=9 Hz, H-17β), 3.95 (3H, s, 3′-OMe), 4.82, 4.97 (1H each dd, J=18, 1 Hz, H-21a, b), 5.18 (1H, d, J=3 Hz, H-1′), 5.19 (1H, d, J=8 Hz, H-1‴), 5.32 (1H, d, J=8 Hz, H-1″), 6.12 (1H, d, J=1 Hz, H-22). ^{13}C-NMR δ (ppm) (pyridine-d_5): 30.3 (C-1), 26.8, 27.1 (C-2, C-6), 73.7, 73.8 (C-3, C-2′), 31.0 (C-4, C-15), 36.8 (C-5), 21.5 (C-6), 41.7 (C-7), 36.0 (C-9), 35.4 (C-10), 20.6 (C-11), 31.6 (C-12), 49.3 (C-13), 85.2 (C-14), 24.9 (C-16), 48.9 (C-17), 18.5 (C-18, C-6′), 23.8 (C-19), 172.7 (C-20), 74.1 (C-21), 116.6 (C-22), 174.2 (C-23), 98.5 (C-1′), 85.3 (C-3′), 81.8 (C-4′), 67.3 (C-5′), 61.0 (3′-OMe), 104.9 (C-1″), 74.7 (C-2″), 76.5 (C-3″), 81.8 (C-4″), 76.3 (C-5″), 62.4 (C-6″), 104.6 (C-1‴), 75.2 (C-2‴), 78.4 (C-3‴), 71.6 (C-4‴), 78.2 (C-5‴), 62.5 (C-6‴). All signals of **2** taken in CD_3OD were in good agreement with those in the literature.[5] Compound **2** (5 mg) was dissolved in 20% EtOH (2 ml) and was shaken with cellulase (Sigma Chem. Co., Ltd.) (5 mg) at 38 °C for 5 h. The mixture was extracted with BuOH and the BuOH extract was examined by TLC and HPLC in parallel with authentic **5** [TLC: solv. 1, Rf 0.65 (**5**, 0.65). HPLC: solv. 30% CH_3CN–H_2O, 1 ml/min, t_R 7.6 min (**5**, 7.6 min)].

17α-Digitoxigenin β-Gentiobiosyl-(1→4)-α-L-thevetoside (3) and Enzymic Hydrolysis of 3——A solid, $[\alpha]_D^{28}$ −55.2° (c=1.3, MeOH). FAB-MS m/z: 881 $(M+Na)^+$. ^{1}H-NMR δ (ppm) (pyridine-d_5): 0.85, 1.19 (3H each, s, H-18, H-19), 1.78 (3H, d, J=6 Hz, H-6′), 3.42 (1H, t, J=9 Hz, H-17β), 4.82, 4.98 (1H each, dd, J=18, 1 Hz, H-21a, b), 5.12 (1H, d, J=3 Hz, H-1′), 5.13 (1H, d, J=8 Hz, H-1‴), 5.32 (1H, d, J=8 Hz, H-1″), 6.13 (1H, d, J=1 Hz, H-22). ^{13}C-NMR δ (ppm) (pyridine-d_5): 30.4 (C-1), 26.8, 27.1 (C-2, C-6), 73.7 (C-3, C-2′), 31.0 (C-4, C-16), 36.9 (C-5), 21.6 (C-7), 41.7 (C-8), 36.1 (C-9), 35.5 (C-10), 20.7 (C-11), 31.7 (C-12), 49.4 (C-13), 85.2 (C-14), 25.0 (C-16), 48.9 (C-17), 18.6 (C-18, C-6′), 23.9 (C-19), 172.9 (C-20), 74.1 (C-21), 116.6 (C-22), 174.2 (C-23), 98.5 (C-1′), 85.5 (C-3′), 81.4 (C-4′), 67.6 (C-5′), 61.0 (3′-OMe), 105.6, 104.8 (C-1″, C-1‴), 75.6, 75.2 (C-2″, C-2‴), 78.4 (×2), 78.3 (C-3″, C-3‴, C-5‴), 71.7, 72.1 (C-4″, C-4‴), 77.1 (C-5″), 70.8 (C-6″), 62.9 (C-6‴). Compound **3** (5 mg) was hydrolyzed with cellulase (5 mg) as described above. The BuOH extract showed the same Rf value and t_R as authentic **5** [TLC: solv. 1, Rf 0.65 (**5**, 0.65). HPLC: solv. 30% CH_3CN–H_2O, 1 ml/min, t_R 7.6 min (**5**, 7.6 min)].

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