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Studies on *Cerbera*. IV.[1] Polar Cardenolide Glycosides from the Leaves of *Cerbera odollam* and *Cerbera manghas*

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Glucos-3-ulosyl-thevetosides of 17α-digitoxigenin and 17α-tanghinigenin were obtained from air-dried leaves of *Cerbera manghas* and *C. odollam*. From fresh leaves, oleagenin glucosyl-thevetoside and digitoxigenin gentiotriosyl-thevetoside were isolated besides known glycosides, glucosyl-thevetosides of digitoxigenin and tanghinigenin The difference between the cardenolide glycosides of the air-dried leaves and of the fresh leaves, and the glycosides patterns in the two species are discussed

Keywords——*Cerbera odollam*; *Cerbera manghas*; Apocynaceae; cardenolide, 17α-cardenolide glucos-3-ulosyl-thevetoside; cerleaside B; oleagenin glucosyl-thevetoside; digitoxigenin gentiotriosyl-thevetoside

In the preceding paper of this series, we described the cardenolide monoglycosides from the air-dried leaves of *Cerbera odollam* GAERTN. and *C. manghas* L., and showed the presence of oleagenin α-L-thevetoside (cerleaside A), 8β-hydroxy-17β- and 17α-digitoxigenin α-L-thevetosides (17β- and 17α-cerdollaside), and 17β- and 17α-digitoxigenin α-L-acofriosides (17β- and 17α-solanoside), together with the four major monosides, 17β- and 17α-neriifolin and 17β- and 17α-deacetyltanghinin.[1] Since the ratio of 17α-cardenolide monosides to the corresponding 17β-isomers was larger in the air-dried leaves, we investigated the presence of the 17α-isomers in the polar glycoside fractions of the air-dried leaves and the fresh leaves. This paper deals with the isolation from the leaves and the structure determinations of new polar glycosides, tentatively designated as compounds **1**—**4**, as well as known biosides,[2] the β-D-glucosyl-(1→4)-α-L-thevetosides of 17β- and 17α-digitoxigenin (**5** and **6**, respectively) and of 17β- and 17α-tanghinigenin (**7** and **8**, respectively), and the known triosides,[2] gentiobiosyl-(1→4)-α-L-thevetosides of digitoxigenin (**9**) and tanghinigenin (**10**).

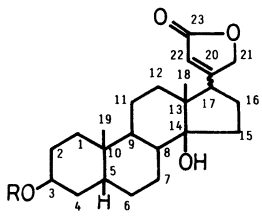
The extraction and isolation of the polar glycosides from the air-dried leaves and stems were carried out as described previously.[1] The benzene and $CHCl_3$ extractives from the MeOH percolate mainly contained the monosides. The biosides and triosides in the BuOH extractives were fractionated by chromatography on columns of one or more of MCI-gel, octadecyl silica and silica gel. The MeOH homogenate from the fresh leaves was eluted with MeOH repeatedly and the MeOH eluate was treated as described for the air-dried leaves. Yields of the biosides and triosides are presented in Table I.

Compounds **1** and **2** were isolated from the air-dried leaves. Compound **1** afforded an $M^+ + Na$ peak at *m/z* 717.347, two mass units smaller than those of **5** and **6**, in the fast atom bombardment (FAB)-mass spectrum (MS), and the molecular formula was determined to be $C_{36}H_{54}O_{13}$. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed 36 carbon signals, of which 30 signals coincided with those of the 17α-digitoxigenin α-L-thevetoside moiety of **6**. Of the remaining 6 signals, one carbonyl carbon signal at δ 207.7, one

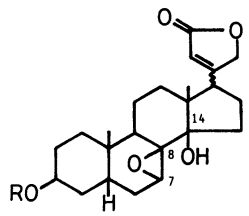
TABLE I. Yields of Polar Cardenolide Glycosides from the Air-Dried Leaves and Fresh Leaves (mg)

Plant materials Date of harvest, place, weight	Biosides							Triosides		Tetraoside
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**4**
Air-dried leaves[a]										
Cerbera odollam July, 1986, Singapore, 0.4 kg	35	4	t[b]	t	t	t				
Cerbera manghas March, 1986, Taiwan, 2.7 kg	120	20	3	10	370	13	28	24	25	
Fresh leaves										
Cerbera odollam Jan , 1986, Singapore, 1.2 kg			27	38		11				
Cerbera manghas Feb., 1986, Okinawa, 0.75 kg				26		180		t		
Cerbera manghas Aug., 1986, Fukuoka, 1.8 kg				300				260		5

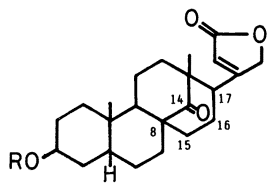
a) Yields of monosides were presented in the preceding paper [1] *b*) "t" means a trace amount



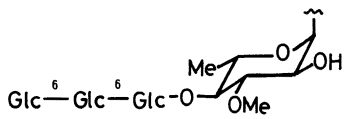
1 : 17α, R=**a**
4 : 17β, R=**c**
5 : 17β, R=**b**
6 : 17α, R=**b**
9 : 17β, R=**d**



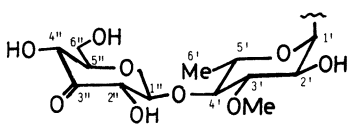
2 : 17α, R=**a**
7 : 17β, R=**b**
8 : 17α, R=**b**
10 : 17β, R=**d**



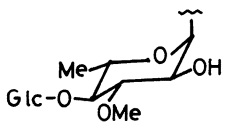
3 : R=**b**



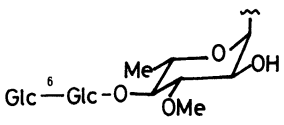
c



a



b



d

Chart 1

primary carbinol carbon signal at δ 62.4 and an anomeric carbon signal at δ 105.9 suggested the presence of a hexosulosyl moiety. A doublet at δ 5.45 in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum was ascribable to an anomeric proton (H-1″). All the protons due to the hexosulose were assigned by means of ^{1}H–^{1}H COSY experiments. The coupling constants between H-1″ and H-2″, and H-4″ and H-5″ were 8 and 10 Hz, respectively, suggesting the relation of H-1″/H-2″ and H-4″/H-5″ to be diaxial in both cases. The terminal sugar was therefore considered to be β-D-glucos-3-ulose which is linked to the 4-hydroxyl of L-thevetose, based on the downfield shift of the C-4 signal of L-thevetose in the ^{13}C-NMR spectrum in comparison with that of 17α-digitoxigenin α-L-thevetoside.[1]

TABLE II ^{1}H Chemical Shifts of **1**, **2**, **3** and **4**, δ (ppm) from Tetramethylsilane in Pyridine-d_5 (J/Hz in Parentheses)

	1	**2**	**3**	**4**
H-18,19	0 85, 1 19	0 99, 1 21	0 72, 0.90	0 82, 1.03
H-21	4.82, 4 98	4.78, 4 93	4 73, 4 83	5.03, 5 31
	(dd, 18, 1)	(dd, 18, 2)	(dd, 18, 2)	(d, 18)
H-22	6.12	6.10	5 89	6.14
	(d, 1)	(d, 2)	(d, 2)	(br s)
H-3α	4.16	4 06	4.09	4.11
	(br s)	(br s)	(br s)	(br s)
H-17	3 42	3 26	2 98	2 81
	(t, 9)	(t, 9)	(br d, 7)	(dd, 5, 9)
H-1′	5.19	5 17	5 16	5 12
	(d, 4)	(d, 4)	(d, 4)	(d, 4)
H-2′	4.00	3 99	4 00	
	(dd, 9, 4)	(dd, 9, 4)	(dd, 9, 4)	
H-3′	4 10	4.09	4 06	
	(t, 9)	(t, 9)	(t, 9)	
H-4′	3 92	3 91	3 90	
	(t, 9)	(t, 9)	(t, 9)	
H-6′	1 70	1 69	1 67	1.74
	(d, 6)	(d, 6)	(d, 6)	(d, 6)
3′-OMe	3.89	3 88	3 94	3 98
H-1″	5.45	5 48	5 35	5.31
	(d, 8)	(d, 8)	(d, 8)	(d, 8)
Others	4.75	4.74	2 68	5.07, 5.08
	(H-2″, dd, 8, 1)	(H-2″, dd, 8, 1)	(H-9, m)	(H-1″, H-1‴, d, 8)
	4.96	4 97	4 37	
	(H-4″, dd, 10, 1)	(H-4″, dd, 9, 3)	(H-6″a, dd, 12, 5)	
	3.86	4 43	4 54	
	(H-5″, br d, 10)	(H-6″a, dd, 12, 4)	(H-6″b, dd, 12, 2)	
	4.43	4 52		
	(H-6″a, dd, 12, 4)	(H-6″b, dd, 12, 1)		
	4.52	3.39		
	(H-6″b, dd, 12, 2)	(H-7α, d, 5)		

Reduction of **1** with $NaBH_4$ afforded two products, of which one product (**1**-1) was identical with **6**. The other product (**1**-2) was characterized as the β-D-allopyranosyl-α-L-thevetoside of 17α-digitoxigenin by a comparison of the ^{13}C-NMR signals with those of the known allopyranoside,[3] and all the proton signals of the sugar moieties were confirmed by ^{1}H–^{1}H COSY experiments.

Compound **2** was isolated in a small amount. The FAB-MS showed the M^++Na peak at m/z 731.325, suggesting **2** to be a dehydro derivative of **7** or **8**. The structure was assigned as 17α-tanghinigenin β-D-glucos-3-ulosyl-(1→4)-α-L-thevetoside on the basis of ^{1}H- and ^{13}C-NMR comparisons with **1**, **8**, and 17α-tanghinigenin α-L-thevetoside.

Compound **3** was isolated from the MeOH homogenate of the fresh leaves of *C. odollam* as one of the major biosides. The characteristic staining of oleagenin with diluted H_2SO_4 reagent was obtained on a thin layer chromatography (TLC) plate. By ^{1}H- and ^{13}C-NMR analysis, the aglycone and sugar moieties were identified as oleagenin and β-D-glucosyl-(1→4)-α-L-thevetose. On enzymic hydrolysis, **3** afforded cerleaside A (oleagenin α-L-thevetoside) and glucose. Compound **3** is therefore oleagenin β-D-glucosyl-(1→4)-α-L-thevetoside and was named cerleaside B.

Compound **4** was obtained from fresh leaves of *C. manghas* as the most polar glycoside.

TABLE III ^{13}C Chemical Shifts of the Polar Cardenolide Glycosides, δ (ppm) from Tetramethylsilane in Pyridine-d_5

	6	1	2	3	9	4
C-1	30.3	30 3	32.0[a]	29 1[a]	30.2	30.2
C-2	26.8[a]	26 8[a]	27.3	26 8[b]	26.8[a]	26.8[a]
C-3	73.7	73.8	72.9	73 4	73.6	73.7
C-4	31 0	31 0	32 2[a]	30 2[a]	31 0	31 0
C-5	36 8	36 8	34 1	37 1	36 8	36 8
C-6	27.1[a]	27 1[a]	28 2	26 9[b]	27 1[a]	27 1[a]
C-7	21.6	21.6	50.9	24.4	21 5[b]	21 5[b]
C-8	41.7	41.7	64.4	47.4[c]	41.9	41.9
C-9	36.0	36.0	32.2	45.9	35.8	35 9
C-10	35.4	35 4	33.9	37 5	35 5	35 5
C-11	20.6	20 6	20 0	21 3	22.0[b]	21 9[b]
C-12	31 7	31 7	33.6	43 9	39 9	39 9
C-13	49 4	49 4	51.7	48 8[c]	50.1	50 1
C-14	85 2	85.2	82.1	221.2	84 6	84.6
C-15	31 0	31.0	32.8[a]	42.5	33.2	33 2
C-16	24.9	25.0	26.3	32.2	27.3[a]	27.3[a]
C-17	48.9	48 9	49.0	52 8	51 5	51 5
C-18	18.6	18 5[b]	19.5	23 3	16 2	16 2
C-19	23.9	23 4	24.3	26 2	23 9	23 9
C-20	172.8	172 8	171 7	171.8	175 9	175.9
C-21	74.1	74 1	74.0	73.3	73.7	73.6
C-22	116.6	116 6	116.9	116 2	117.6	117.6
C-23	174.1	174 1	174.0	173 7	174 4	174 4
C-1′	98.5	98 5	98.5	98 5	98.5	98 5
C-2′	73.7	73 8	73 8[b]	73 7	73 6	73 7
C-3′	85 3	85 3	85 2	85 2	85 4	85 4
C-4′	81.8	81.9	81.9	81 8	81.4	81 5
C-5′	67 4	67 2	67.2	67 4	67.5	67 6
C-6′	18.6	18.4[b]	18.6	18.6	18.7	18 7
3′-OMe	61 0	61 1	61 1	61 0	61 0	60 9
C-1″ (1‴,1⁗)	105.1	105 9	105 9	105 1	104 8, 105 6	104 8, 105.5 (×2)
C-2″ (2‴,2⁗)	75.8	78 7	78 7	75 7	75 2, 75 5	75 1, 75 2, 75.5
C-3″ (3‴,3⁗)	78.3[b]	207 7	207.7	78.3	78.4 (×2)[c]	78 3 (×3)[c]
C-4″ (4‴,4⁗)	72.1	73 8	73.7[b]	72 1	71.7, 72.1	71 5, 71.6, 72.1
C-5″ (5‴,5⁗)	78.2[b]	78 4	78.4	78 2	77.1, 78 3[c]	77.0 (×2), 78.2[c]
C-6″ (6‴,6⁗)	63.1	62 4	62.4	63 1	70 8, 62.8	70 1, 70.7, 62.8

a—*c*) Signal assignments marked *a*), *b*) or *c*) in each column may be reversed

The molecular formula was considered to be $C_{48}H_{76}O_{23}$, based on the $M^+ + Na$ peak at *m/z* 1043, suggesting **4** to be a tetraoside. The presence of three hexoses was shown by the fragment peaks at *m/z* 857, 695, and 533 together with the $(M-1)^-$ peak at *m/z* 1019 in the negative FAB-MS. In the ^1H-NMR spectrum, four anomeric proton signals were observed at δ 5.07 (d, $J=8$ Hz), 5.08 (d, $J=8$ Hz), 5.12 (d, $J=4$ Hz), and 5.31 (d, $J=8$ Hz). The signal at δ 5.12 was assigned to L-thevetose. The ^{13}C-NMR spectrum also showed the presence of a gentiobiosyl-neriifolin (thevetin B) moiety. Since the carbon signals due to the terminal glucose unit were also observed and the carbon signals at δ 70.7 and 70.1 were both assignable to the glucosylated C-6 of glucose, the linkages between the three glucose units were determined to be $1\rightarrow6\beta$. Finally, **4** was subjected to enzymic partial hydrolysis to yield **5** and **9**, and the structure was thus confirmed.

While monosides and biosides of 17α-cardenolides were present as the major glycosides

in air-dried leaves,[1] no biosides or triosides of 17α-cardenolides were isolated from fresh leaves, indicating that isomerization at C-17 and deglucosylation had occurred during the air-drying procedure.

It should be noted that glycosides having the hexos-3-ulosyl moiety were seen only as 17α-digitoxigenin and 17α-tanghinigenin glycosides in the air-dried leaves. The oxidation of the 3-hydroxyl group of the terminal glucose in the biosides is thought to occur during the air-drying procedure and appears to prevent further enzymic transformation into monosides.

Whilst cerleaside A occurs in the air-dried leaves of *Cerbera odollam*, cerleaside B was obtained as one of the major biosides from the fresh leaves, confirming that oleagenin is one of the natural cardenolides in *Cerbera*. This is the first report of the isolation of a cardenolide tetraoside having a gentiotriosyl moiety. The amount of tanghinigenin glycosides in *C. manghas* varied from sample to sample. No glucosyl-cerdollaside was obtained from the fresh leaves, probably because of its low content.

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 (bottom layer); solv. 2, EtOAc–MeOH–H_2O (top layer). Spots on the TLC plate were detected by spraying with 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.

Extraction and Isolation of 1 and 2 from the Air-Dried Leaves——Isolation of the monosides from the air-dried leaves of *C. odollam* (collected in Singapore, July, 1986) (400 g) and *C. manghas* (collected in Taiwan, March, 1986) (2.7 kg) has been described in the preceding paper.[1] After extraction of the monosides with benzene and $CHCl_3$, the H_2O layer was concentrated *in vacuo* to half its original volume and extracted with BuOH. The BuOH extractives were passed through an MCI-gel (Mitsubishi CHP 20P) column and the column was eluted with H_2O–MeOH, gradually increasing the MeOH concentration to 100%. The eluates from 60% to 80% MeOH were combined and the solvent was evaporated off *in vacuo*. The residue was chromatographed on a silica gel column with solv. 1 (7:2:1—7:3:1) to isolate biosides (**1**—**8** from *C. manghas*, **1**—**7** from *C. odollam*) and triosides (**9** and **10** from *C. manghas*) (Table I).

17α-Digitoxigenin β-D-Glucos-3-ulosyl-(1→4)-α-L-thevetoside (1) and $NaBH_4$ Reduction of 1——A solid, $[\alpha]_D^{27}$ −93.5° (c=0.40, MeOH). FAB-MS m/z: 717.347 (Calcd for $C_{36}H_{54}O_{13}$+Na, 717.346). A solution of **1** (25 mg) in EtOH (2 ml) was stirred at room temperature, and $NaBH_4$ (10 mg) was added portionwise. After being stirred for 20 min, the mixture was diluted with H_2O and extracted with BuOH. The BuOH extract was chromatographed on a silica gel column with solv. 1 (7:2:1) to isolate two products as solids, (**1**-1, 6 mg; **1**-2, 6 mg). In a comparison of the ^{1}H-NMR spectra, all signals of **1**-1 and of **6** were identical. Compound **1**-2: $[\alpha]_D^{24}$ −88.3° (c=0.30, MeOH). ^{1}H-NMR δ (ppm): 0.86, 1.19 (3H, each s, H-18, H-19), 3.42 (1H, t, J=9 Hz, H-17), 4.14 (1H, br s, H-3α), 4.82, 4.97 (1H each, dd, J=18, 1 Hz, H-21a, b), 6.12 (1H, d, J=1 Hz, H-22), 5.17 (1H, d, J=4 Hz, H-1′), 3.99 (1H, dd, J=9, 4 Hz, H-2′), 4.08 (1H, t, J=9 Hz, H-3′), 3.88 (1H, t, J=9 Hz, H-4′), 4.27 (1H, m, H-5′), 1.69 (3H, d, J=6 Hz, H-6′), 3.90 (3H, s, 3′-OMe), 5.73 (1H, d, J=8 Hz, H-1″), 3.96 (1H, dd, J=8, 3 Hz, H-2″), 4.73 (1H, t, J=3 Hz, H-3″), 4.21 (1H, dd, J=3, 9 Hz, H-4″), 4.44 (1H, m, H-5″), 4.36 (1H, dd, J=5, 11 Hz, H-6″a), 4.49 (1H, dd, J=1, 11 Hz, H-6″b).

17α-Tanghinigenin β-D-Glucos-3-ulosyl-(1→4)-α-L-thevetoside (2)——A solid, $[\alpha]_D^{27}$ −69.5° (c=0.20, MeOH). FAB-MS m/z: 731.325 (Calcd for $C_{36}H_{52}O_{14}$+Na, 731.325).

Isolation of 3 from the Fresh Leaves of *C. odollam*——Fresh leaves (collected in Singapore, Jan., 1986) (1.2 kg) were homogenized with MeOH. The homogenate was packed in a column and was exhaustively eluted with MeOH. The total MeOH extract was concentrated *in vacuo* to 2 l and the deposit was filtered off. The filtrate was diluted with H_2O and extracted with BuOH. The BuOH extract was passed through an MCI gel column and the column was eluted with H_2O–MeOH with increasing MeOH concentration. The fractions containing biosides and triosides were combined and subjected to silica gel column chromatography with solv. 1 (7:2:1—7:3:2) and solv. 2 (6:1:5—4:1:3) to isolate **3**, **5** and **7** (Table I).

Oleagenin β-D-Glucosyl-(1→4)-α-L-thevetoside (Cerleaside B) (3) and Enzymic Hydrolysis of 3——Prisms from MeOH, mp 250—253 °C, $[\alpha]_D^{24}$ −30.5° (c=0.32, MeOH). FAB-MS m/z: 717.346 (Calcd for $C_{36}H_{54}O_{13}$+Na: 717.346). Compound **3** (5 mg) was dissolved in 25% EtOH (2 ml) and shaken with snail digestive juice (acetone-dried powder) (3 mg) for 5 h at 38 °C. The mixture was then diluted with H_2O and extracted with BuOH. The BuOH extract showed a spot and peak corresponding to cerleaside A on TLC (solv. 1 (7:2:1), Rf 0.80) and HPLC (solv. 30% CH_3CN–H_2O, 1 ml/min, t_R 14.2), respectively. Glucose was detected from the H_2O layer by TLC.

Isolation of 4 from the Fresh Leaves of *C. manghas*——Fresh leaves of *C. manghas*, cultivated in the greenhouse

of Fukuoka University, were harvested in Aug., 1986 (1.8 kg), and were homogenized with MeOH. The MeOH homogenate was filtered and the filtrate was treated in the same manner as described above. Compound **4** (5 mg) was isolated along with **5** and **9** (Table I).

Fresh leaves of *C. manghas* collected in Okinawa in Feb., 1986 (0.75 kg) were homogenized in the same manner as described above. Compounds **5**, **7**, and **9** were isolated (Table I).

Digitoxigenin β-D-Gentiotriosyl-(1→4)-α-L-thevetoside (4) and Enzymic Hydrolysis of 4——A solid, $[\alpha]_D^{27}$ −25.7° (c=0.11, MeOH). FAB-MS m/z: 1043 ($C_{48}H_{76}O_{23}$+Na), negative FAB-MS m/z: 1019 $(M-1)^-$, 857 $(M-Glc-1)^-$, 695 $(857-Glc)^-$, 533 $(695-Glc)^-$. Compound **4** (2 mg) was dissolved in 20% EtOH (1 ml) and shaken with cellulase (Sigma Chem. Co., Ltd.) (1 mg) for 5 h at 38 °C. The mixture was diluted with H_2O and extracted with BuOH. The BuOH extract showed two spots identical with **5** and **9** on TLC (solv. 1 (7 : 3 : 1) *Rf* **5**; 0.66, **9**; 0.23) and HPLC (solv. 30% CH_3CN–H_2O, 1 ml/min, t_R **5**; 12.2, **9**; 6.3).

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