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C-NOR-D-HOMO-CARDENOLIDE GLYCOSIDES FROM *THEVETIA NERIIFOLIA*

FUMIKO ABE, TATSUO YAMAUCHI* and TOSHIHIRO NOHARA†

Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-01, Japan; †Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan

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Key Word Index—*Thevetia neriifolia*; Apocynaceae; leaves; cardenolide, *C*-nor-D-homo-cardenolide; thevetioside.

Abstract—Along with known digitoxigenin α-L-thevetoside and α-L-acofrioside, the corresponding glycosides of a *C*-nor-D-homocardenolide were isolated from the air-dried leaves of *Thevetia neriifolia* and their structures determined by spectral and chemical methods. Triosides of the *C*-nor-D-homo-cardenolide were obtained from the methanol extract of the fresh leaves.

INTRODUCTION

Thevetia neriifolia Juss. is widely planted as a garden tree in tropical districts. Cardiac glycosides from the seed kernels of this plant have been investigated and α-L-thevetosides, glucosyl- and gentiobiosyl-α-L-thevetosides of digitoxigenin [1], cannogenin [2, 3], cannogenol [4], and uzarigenin [5] reported. In the course of our investigations on the constituents of Apocynaceae plants, the leaves and stems of *T. neriifolia* were examined. We deal with the isolation and the structure determination of *C*-nor-D-homo-cardenolide glycosides along with the L-thevetoside and L-acofrioside series of digitoxigenin from the leaves.

RESULTS AND DISCUSSION

Seven cardiac glycosides (**1**–**7**) were isolated from the air-dried leaves. Compounds **1** and **2** were identified as digitoxigenin α-L-thevetoside (neriifolin) [1] and digitoxigenin α-L-acofrioside (solanoside) [6, 7], respectively, based on FAB mass, ^{1}H and ^{13}C NMR spectra and direct comparisons with the authentic samples.

The FAB mass spectrum of **3** afforded a $[M+Na]^+$ peak at *m/z* 555.294, molecular formula $C_{30}H_{44}O_8$, 2H less than those of **1** and **2**. The signals due to the sugar moiety in the ^{1}H and ^{13}C NMR spectra suggested **3** to be an α-L-thevetoside. The unsaturated five-membered lactone ring at C-17 was revealed by signals due to H-21 (δ4.81 and 4.85) and H-22 (δ6.23). However, only one angular methyl signal was observed (^{1}H δ0.81; ^{13}C δ22.7), and it was assignable to C-19 in a 5β-cardenolide based on the chemical shifts of C-1–C-7 and C-10 which were almost the same as those of **1**. The assignments of the C-5 and C-9 signals were confirmed by the cross peaks of H-19/C-5, C-9, and C-1, and H-3/C-5 and C-1 in the COLOC spectrum.

The presence of an exomethylene group was revealed by the signals of two carbons (δ110.4 *t*, 147.6 *s* and two olefinic protons (δ5.10 *br s*, 5.15 *br s*). In the COLOC

*Author to whom correspondence should be addressed.

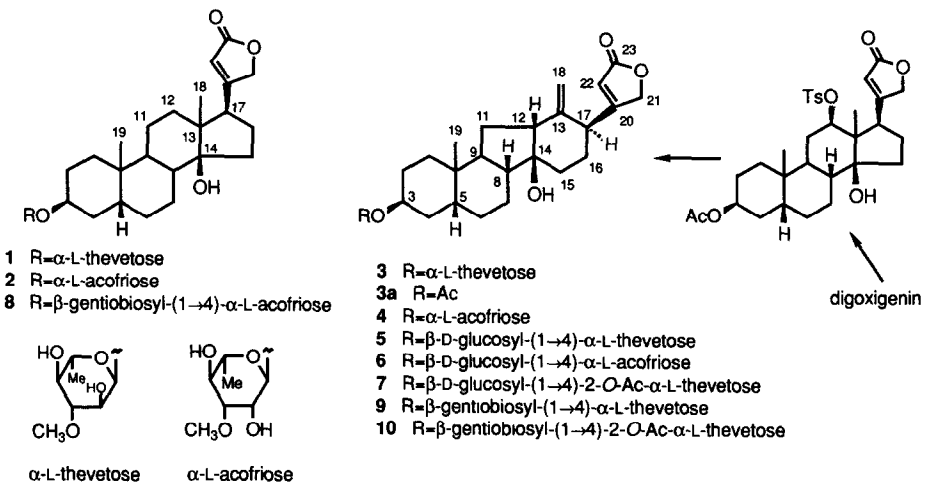

Table 1. ^{1}H NMR chemical shifts of cardiac glycosides [δ (ppm) in pyridine-d_5 (400 MHz)]

H	1	3	3a	4	5	6	7	8	9	10
3	4.17 *br s*	3.99 *br s*	5.07 *br s*	4.01 *br s*	4.21 *br s*	3.96 *br s*	4.22 *br s*			
9		2.65 *m*	2.59 *m*	2.66 *m*	2.64 *m*	2.65 *m*	2.65 *m*		2.64 *m*	2.65 *m*
12		2.23 *br t*	2.25 *br t*	2.25 *br t*	2.25 *br t*	2.25 *br t*	2.24 *br t*		2.24 *br t*	2.26 *br t*
		(9)*†	(10)	(9)	(9)	(9)	(9)		(9)	(9)
17	2.80 *dd*	3.47 *br d*	3.48 *br d*	3.48 *br d*	3.48 *br d*	3.48 *br d*	3.49 *br d*	2.80 *dd*	3.48 *br d*	3.49 *br d*
	(9, 5)	(6)‡	(6)	(6)	(6)	(6)	(6)	(9, 5)	(5)	(6)
18	1.02 *s*	5.10 *br s*	5.11 *br s*	5.11 *br s*	5.10 *br s*	5.11 *br s*	5.11 *br s*	1.04 *s*	5.11 *br s*	5.11 *br s*
		5.15 *br s*‡	5.15 *br s*	5.15 *br s*	5.15 *br s*	5.16 *br s*	5.16 *br s*		5.15 *br s*	5.16 *br s*
19	0.83 *s*	0.81 *s*§	0.90 *s*	0.87 *s*	0.80 *s*	0.87 *s*	0.90 *s*	0.89 *s*	0.79 *s*	0.90 *s*
21	5.02 *dd*	4.81 *dd*	4.82 *br d*	4.81 *br d*	4.81 *br d*	4.82 *br d*	4.82 *br d*	5.03 *dd*	4.81 *br d*	4.82 *br d*
	(18, 1)	(18, 1)	(18)	(18)	(18)	(18)	(18)	(18, 1)	(18)	(18)
	5.30 *dd*	4.85 *dd*	4.86 *br d*	4.85 *br d*	4.85 *br d*	4.86 *br d*	4.86 *br d*	5.31 *dd*	4.85 *br d*	4.86 *br d*
	(18, 1)	(18, 1)	(18)	(18)	(18)	(18)	(18)	(18, 1)	(18)	(18)
22	6.12 *br s*	6.23 *br s*†	6.22 *dd*	6.23 *br s*	6.23 *br s*	6.23 *br s*	6.23 *br s*	6.13 *br s*	6.24 *br s*	6.24 *br s*
			(3, 1)							
1′	5.23 d_a(4)	5.17 *d* (4)		5.35 *d* (1)	5.11 *d* (4)	5.31 *d* (1)	5.29 *d* (4)	5.32 *d* (1)	5.06 (4)	5.27 *d* (4)
2′	4.05 *dd*	4.03 *dd*		4.53 *br s*		4.48 *br s*	5.00 *dd*			4.95 *dd*
	(9, 4)	(9, 4)					(9, 4)			(10, 4)
6′	1.60 *d* (6)	1.56 *d* (6)		1.61 *d* (6)	1.63 *d* (6)	1.63 *d* (6)	1.56 *d* (6)	1.77 *d* (6)	1.74 *d* (6)	1.69 *d* (6)
−OMe	3.83 *s*	3.83 *s*		3.55 *s*	3.95 *s*	3.54 *s*	3.83 *s*	3.53 *s*	3.93 *s*	3.82 *s*
1″					5.33 *d* (8)	5.24 *d* (8)	5.30 *d* (8)	5.22 *d* (8)	5.29 *d* (8)	5.24 *d* (8)
1‴								5.10 *d* (8)	5.12 *d* (8)	5.12 *d* (8)
Others	3.99 *t* (9)	1.53 *td* (12, 1)	2.06	1.53 *td* (12, 1)			2.13 *s*			2.12 *s*
	(H-3′)	(H-8)*§	(−OAc)	(H-8)			(−OAc)			(−OAc)
	3.65 *t* (9)	3.96 *t* (9)		3.92 *dd* (9, 3)						
	(H-4′)	(H-3′)		(H-3′)						
	4.30 *dq*	3.62 *t* (9)		4.25 *t* (9)						
	(9, 6) (H-5′)	(H-4′)		(H-4′)						
		4.27 *dq* (9, 6)		4.22 *dq* (9, 6)						
		(H-5′)		(H-5′)						

Coupling constants (J in Hz) are given in parentheses.

*†‡§Cross peaks were observed between the signals marked in the 2D-NOESY spectrum.

spectrum, the olefinic protons showed cross peaks to C-17 and the other methine carbon which can be assigned to C-12, suggesting that the exomethylene group was located on C-18/C-13 in a *C*-nor-D-homo-framework. The sequence from H-12 to H-9 through one methylene group (H-11a, b) was confirmed by ^1H–^1H COSY which also indicated the presence of a five-membered ring C. The coupling pattern of C-12 (*d*) was consistent with a six-membered ring D.

In order to confirm the structure, the *C*-nor-D-homocardenolide was prepared from digoxigenin-3-*O*-acetyl-12-tosylate by heating in pyridine [8]. The *C*-nor-D-homocardenolide 3-acetate (**3a**), thus obtained, showed identical chemical shifts with those of the aglycone moiety of **3**, except for the signals due to ring A (Tables 1 and 2). In the NOESY of **3**, cross peaks were observed at H-12/H-22, and H-17/H-18a, as well as H-8/H-19 and H-12, suggesting the half chair (sofa) conformation of ring D, raising C-17. Consequently, the aglycone of **3** is 3β,14-dihydroxy-14(13→12)*abeo*-5β,12β,14β-carda-13(18),20(22)-dienolide. Compound **3** is named thevetioside A.

Table 2. ^{13}C NMR chemical shifts of cardiac glycosides [δ (ppm) in pyridine-d_5 (100 MHz)]

C	1	3*	3a	4*	5	6	7	8	9	10
1	30.3	32.5	32.3	32.5	32.5	32.5	32.5	30.0	32.5	32.5
2	26.9[a]	26.9	25.2	26.9	26.9	26.9	26.9	26.8[a]	26.9	26.9
3	73.7	73.2	70.6	72.1	73.7[a]	72.2[a]	72.8[a]	72.3	73.5[a]	72.7
4	31.0	30.8	31.2	30.5	30.7	30.4	30.3	31.0	30.7	30.3
5	36.8	36.5	37.0	36.8	36.5	36.8	36.7	37.1	36.5	36.7
6	27.1[a]	28.1	27.8	28.2	28.1	28.2	28.1	27.2[a]	28.1	28.1
7	21.5[b]	20.8	20.6	20.8	20.7	20.8	20.8	21.9[b]	20.8	20.8
8	41.9	50.3	50.2	50.3	50.3	50.3	50.3	41.9	50.3	50.4
9	35.8	38.7	38.5	38.7	38.7	38.6	38.6	35.8	38.7	38.6
10	35.5	35.2	35.2	35.3	35.2	35.3	35.3	35.5	35.2	35.3
11	21.9[b]	21.8	21.7	21.8	21.8	21.8	21.8	21.5[b]	21.8	21.8
12	39.8	50.5	50.4	50.5	50.5	50.5	50.5	39.9	50.5	50.5
13	50.1	147.6	147.5	147.6	147.6	147.6	147.6	50.1	147.6	147.6
14	84.6	79.8	79.8	79.8	79.8	79.6	79.8	84.6	79.8	79.8
15	33.2	31.8	31.8	31.8	31.8	31.8	31.8	33.2	31.8	31.8
16	27.1[a]	25.3	25.2	25.3	25.3	25.3	25.2	27.3[a]	25.3	25.2
17	51.5	44.4	44.3	44.4	44.4	44.4	44.4	51.5	44.4	44.4
18	16.2	110.4	110.4	110.4	110.4	110.4	110.4	16.1	110.4	110.4
19	23.8	22.7	22.6	22.8	22.7	22.8	22.9	24.0	22.7	22.9
20	175.9	173.3	173.2	173.3	173.3	173.3	173.3	175.8	173.3	173.3
21	73.6	73.0	73.0	73.0	73.0	73.0	73.0	73.7	73.0	73.0
22	117.6	116.0	116.0	116.0	116.0	116.0	116.0	117.6	116.0	116.0
23	174.4	174.2	174.1	174.2	174.2	174.2	174.2	174.4	174.2	174.2
1′	98.8	98.7	(−OAc)	99.5	98.4	99.2	94.0	99.3	98.4	93.9
2′	73.4	73.4	21.2	68.7	73.6[a]	68.3[b]	75.0	68.4[c]	73.6[a]	75.1[a]
3′	85.4	85.4	170.1	82.8	85.3	82.6	82.0	82.6	85.4	82.1
4′	76.6	76.5		72.4	81.8	79.5	81.1	78.8	81.4	80.9
5′	68.9	68.9		69.9	67.3	68.2[b]	67.1	68.2[c]	67.5	67.2
6′	18.5	18.4		18.5	18.5	18.5	18.2	18.7	18.6	18.3
−OMe	60.5	60.4		57.0	61.0	56.6	61.0	56.6	60.9	61.0
1″					105.0	105.5	104.8	105.5	104.8	104.6
2″					75.7	76.0	75.5	75.2[d]	75.2[b]	75.2[a]
3″					78.3[b]	78.2[c]	78.3[b]	78.3	78.3	78.3
4″					72.1	71.9[a]	72.1[a]	71.8	72.0	72.1
5″					78.2[b]	78.0[c]	78.2[b]	77.0	77.0	77.0
6″					63.1	62.9	63.1	70.5	70.7	70.8
1‴							(−OAc)	105.2	105.5	105.6
2‴							20.8	75.8[d]	75.5[b]	75.3[a]
3‴							170.4	78.4	78.4	78.4
4‴								71.7	71.7	71.7
5‴								78.4	78.4	78.4
6‴								62.8	62.8	62.8
										(−OAc)
										20.8
										170.3

*Signal assignments were based on ^{13}C–^1H COSY spectra and in case of **3** also on COLOC spectrum.

[a–d] Interchangeable within the same column.

Compounds **4**–**7** retain the same aglycone, thevetiogenin, as present in **3**, and their sugar moieties were characterized as α-L-acofrioside, β-D-glucosyl-(1→4)-α-L-thevetoside, β-D-glucosyl-(1→4)-α-L-acofrioside and β-D-glucosyl-(1→4)-2′-*O*-acetyl-α-L-thevetoside, respectively, based on FAB mass and the ^{13}C NMR spectra (Table 2). Compounds **4**–**7** are named thevetiosides B–E, respectively.

In order to examine whether the *C*-nor-D-homocardenolide is a genuine cardenolide or an artifact transformed from 12-*O*-acyl cardenolides during the harvest to extraction process, the isolation of the original glycosides in the fresh leaves was attempted. The careful elution of the fresh leaves with methanol without homogenization, followed by the reversed phase column chromatography, afforded four polar glycosides, **5** and **8**–**10**, of which **9** was a major glycoside and characterized as thevetiogenin β-gentiobiosyl-α-L-thevetoside (thevetioside F). Compounds, **8** and **10**, were identified as digitoxigenin β-gentiobiosyl-(1→4)-α-L-acofrioside and thevetiogenin β-gentiobiosyl-(1→4)-2′-*O*-acetyl-α-L-thevetoside (thevetioside G), respectively. The minor triosides to be expected in the fresh leaves were obtained as a mixture and identified as digitoxigenin β-gentiobiosyl-(1→4)-α-L--thevetoside (thevetin B) (**11**) and thevetiogenin β-gentiobiosyl-(1→4)-α-L-acofrioside (**12**) by analysis of the ^1H NMR spectra.

Whereas 1 or 2 mol of glucose may be lost during the air-drying procedure, the triosides of *C*-nor-D-homocardenolide were obtained possibly as genuine glycosides from the extract of fresh leaves. Upon comparison of L-thevetosides with L-acofriosides, the former are predominant in the thevetiogenin glycosides while the latter are major in the digitoxigenin glycosides from the leaves.

EXPERIMENTAL

General. Mps: uncorr. NMR: 400 and 100 MHz, TMS as int. standard. The following solvent systems were used, S1: $CHCl_3$–MeOH–H_2O (bottom layer), S2: EtOAc–MeOH–H_2O (top layer).

Plant material. Thevetia neriifolia Juss was cultivated in the greenhouse of Kumamoto University. The air-dried leaves were harvested in August 1989. The fresh leaves were collected in January 1991.

Extraction and isolation of glycosides from the air-dried leaves. Powdered air-dried leaves (1.9 kg) were percolated with MeOH and the MeOH evapd *in vacuo* to 500 ml. To the concentrate, 500 ml of H_2O was added and the mixt. was partitioned with C_6H_6, $CHCl_3$, and then with BuOH. The $CHCl_3$ extract was subjected to CC on a silica gel column with S1 (7:1·1–7:2:1) and S2 (10:1:6–6.1:5). The BuOH layer was passed through a polystyrene column (MCI-gel, CHP-20) and a 80–100% MeOH eluate was then chromatographed on a silica gel column with S1 and S2. The following glycosides were obtained, **1** (neriifolin): 10 mg, **2** (solanoside): 45 mg, **3**: 45 mg, **4**: 15 mg, **5**: 20 mg, **6**: 11 mg, **7**: 10 mg.

Thevetioside A (**3**). A solid, $[\alpha]_D^{27}$ +20.6° (MeOH; *c* 0.85), FAB-MS *m/z* 555.294 ($C_{30}H_{44}O_8Na$ requires 555.293). Cross peaks in the COLOC spectrum: (^2J) H-17/C-13, 20; H-19/C-10; H-21/C-20; H-22/C-23, (^3J) H-3/C-1, 5; H-16/C-13, 14; H-17/C-12,15,18; H-18/C-12,17; H-19/C-1,5,9; H-22/C-21.

Thevetioside B (**4**) A solid, $[\alpha]_D^{26}$ +43.1° (MeOH; *c* 0.65), FAB-MS *m/z* 555.293 ($C_{30}H_{44}O_8Na$ requires 555.293).

Thevetioside C (**5**) A solid, $[\alpha]_D^{27}$ −7.3° (MeOH; *c* 1.0), FAB-MS *m/z* 717.348 ($C_{36}H_{54}O_{13}Na$ requires 717.346).

Thevetioside D (**6**) A solid, $[\alpha]_D^{26}$ +4.6° (MeOH; *c* 1.5), FAB-MS *m/z* 717.350 ($C_{36}H_{54}O_{13}Na$ requires 717.346).

Thevetioside E (**7**) A solid, $[\alpha]_D^{27}$ −27.4° (MeOH; *c* 0.5), FAB-MS *m/z* 759.357 ($C_{38}H_{56}O_{14}Na$ requires 759.357).

Thevetiogenin 3-acetate (**3a**) Digoxigenin diacetate (170 mg) (mp 223–228°) was partially deacetylated with 2% $KHCO_3$–MeOH at room temp. for 2 days and the 3-acetate mp 242–250° (dec), thus obtained, was tosylated with TsCl and pyridine to afford digoxigenin-3-*O*-acetyl-12-tosylate (80 mg) [mp 126–129°, FAB-MS *m/z*: 587.268 ($C_{32}H_{42}O_8S+1$ requires 587.268)]. Digoxigenin-3-*O*-acetyl-12-tosylate (70 mg) was then refluxed in pyridine for 1 hr to afford **3a** (45 mg). Compound **3a** was a solid, $[\alpha]_D^{26}$ +114.4° (MeOH; *c* 1.3), FAB-MS *m/z* 415.247 ($C_{25}H_{34}O_5+1$ requires 415.248).

Extraction and isolation of glycosides from the fresh leaves. Fresh leaves (180 g) were soaked in MeOH (3 l) and the mixt. allowed to stand for 1 week. The MeOH was removed by decantation and the leaves further percolated with MeOH (2 l). The MeOH solns were combined and concd *in vacuo* to 200 ml. The MeOH soln was worked-up by the same procedure described above and the following glycosides were obtained. Compound **5**: 4 mg, **8** (digitoxigenin β-gentiobiosyl-α-L-acofrioside): 9 mg, **9**: 17 mg, **10**: 11 mg, a mixt. of **11** and **12**: 4 mg.

Thevetioside F (**9**). A solid, $[\alpha]_D^{26}$ −25.2° (MeOH; *c* 0.75), FAB-MS *m/z* 879.397 ($C_{42}H_{64}O_{18}Na$ requires 879.399).

Thevetioside G (**10**). A solid, $[\alpha]_D^{26}$ −29.4° (MeOH; *c* 0.5), FAB-MS *m/z* 921.410 ($C_{44}H_{66}O_{19}Na$ requires 921.410).

Mixt. of compounds **11** *and* **12** A solid, ^1H NMR δ: (**11**) 0.82, 1.02 (3H each, *s*, H-18, H-19), 1.78 (3H, *d*, *J*=6 Hz, H-6′), 2.80 (1H, *dd*, *J*=9, 5 Hz, H-17), 3.94 (3H, *s*, OMe-3′), 5.12 (1H, *d*, *J*=4 Hz, H-1′), 6.13 (1H, *br s*, H-22). (**12**) 0.86 (3H, *s*, H-19), 1.74 (3H, *d*, *J*=6 Hz, H-6′), 3.48 (1H, *br d*, *J*=5 Hz, H-17), 3.53 (3H, *s*, OMe-3′), 5.10, 5.16 (1H each, *br s*, H-18), 5.32 (1H, *br s*, H-1′), 6.24 (1H, *br s*, H-22).

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