Cardenolide Glycosides from *Thevetia ahouai* (LINN.) A.DC.

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Twenty cardenolide glycosides were obtained from the leaves, wood and twigs of *Thevetia ahouai* (LINN.) A.DC., Apocynaceae. Among these, four compounds were determined to be new combinations of known aglycones and sugars, based on spectral and chemical evidence, and nine compounds were C-nor-D-homo cardenolide glycosides. The new compounds were thevetiogenin 3-*O*-β-gentiobiosyl-(1→4)-α-L-rhamnopyranoside, thevetiogenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-acofriopyranoside and digitoxigenin 3-*O*-β-D-glucopyranosyl-(1→4)-2′-D-acetyl-α-L-thevetopyranoside.

Key words *Thevetia ahouai*; Apocynaceae; C-nor-D-homo cardenolide; thevetiogenin; digitoxigenin; cardenolide glycoside

Thevetia ahouai (LINN.) A.DC., Apocynaceae[1] is distributed throughout Central America. Cytotoxic cardenolide glycosides from this plant have been investigated and neriifolin, 3′-*O*-methylevomonoside and 2′-acetylneriifolin have been reported.[2] Some C-nor-D-homo cardenolide glycosides were isolated by Abe *et. al.* from the leaves of *Thevetia neriifolia* which is one of the most studied plants in this genus.[3] In this paper, we obtained twenty cardenolide glycosides including four new compounds.

Fresh leaves, wood and twigs were extracted with 70% EtOH or Et_2O. These 70% EtOH extracts were processed by the method described in the Experimental section. Twenty cardenolide glycosides (**1**—**20**) were isolated.

Compounds **1**—**3**, **5**, **6**, **8** and **9** were known thevetiogenin glycosides, C-nor-D-homo cardenolide glycosides. They were identified as thevetioside I[3b] (**1**), thevetioside A[3a] (**2**), thevetioside B[3a] (**3**), thevetioside C[3a] (**5**), thevetioside D[3a] (**17**), thevetioside F[3a] (**8**) and thevetioside H[3b] (**9**), based on comparison of spectral data NMR, MS and specific optical rotation with values in the literatures.

Compounds **10**—**13** and **17**—**20**, were known digitoxigenin glycosides. They were identified as evomonoside[4] (**10**), neriifolin[5] (**11**), solanoside[5] (**12**), thevetin B[6,7] (**18**) and digitoxigenin 3-*O*-β-gentiobiosyl-(1→4)-α-L-acofrioside[3a] (**19**). The component sugars of **13** and **17** were identified as 2′-*O*-acetyl-α-L-thevetose and β-gentiobiosyl-(1→4)-α-L-rhamnose, respectively, based on the ^{1}H- and ^{13}C-NMR spectra. So **13** and **17** were characterized as cerberin[7] and evonoside,[8] respectively. Compounds **14** and **20** were determined to be digitoxigenin 3-*O*-β-D-glucosyl-(1→4)-α-L-thevetoside[3b] and digitoxigenin 3-*O*-β-gentiobiosyl-(1→4)-2′-*O*-acetyl-α-L-thevetoside,[3c] respectively, by comparison of the NMR spectra with glycosides having corresponding sugar moieties.[3a]

Compound **7** was obtained as an amorphous powder, and afforded a $[M+Na]^+$ ion peak at *m/z* 865 ($C_{41}H_{62}O_{18}Na$) in the positive-ion FAB-MS. The ^{1}H-NMR signals of a methylene proton at δ 4.83 (2H, br s) and of an olefinic proton at δ 6.24 (1H, d, J=1.5 Hz) suggested the existence of a butenolactone ring. Moreover, the signals of exomethylene protons and carbons were observed at δ 5.11 (1H, br s) and δ 5.15 (1H, br s) in the ^{1}H-NMR spectra and at δ 110.5 and δ 147.7 in the ^{13}C-NMR spectra. These signals suggested the aglycone of **7** to be thevetiogenin, C-nor-D-homo cardenolide.[3a,3b] In the ^{1}H- and ^{13}C-NMR spectra, **7** exhibited three anomeric proton and carbon signals at δ 5.28 (br s), δ 5.18 (d, J=7.5 Hz), δ 5.08 (d, J=7.5 Hz); and δ 99.5, δ 106.4, δ 105.5, respectively.

Acid hydrolysis gave rhamnose and glucose as the sugar components. An analysis of the sugar peak areas in the gas chromatogram, and the number and δ values of the anomeric proton signals, indicated that the ratio of glucose to rhamnose was 2 : 1.

In the heteronuclear multiple bond connectivity (HMBC) spectra, long-range couplings ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 5.28 (H-1 of rhamnose) and the carbon signal at δ 72.4 or δ 72.5 due to C-3 of the aglycone, between the anomeric proton signal at δ 5.18 (H-1 of glucose) and the carbon signal at δ 84.8 due to C-4 of rhamnose and between the anomeric proton signal at δ 5.08 (H-1 of glucose) and the carbon signal at δ 70.2 due to C-6 of glucose. In the rotating frame nuclear Overhauser effect difference (ROED) spectra, irradiation at δ 5.28 (H-1 of rhamnose), δ 5.18 (H-1 of glucose) and δ 5.08 (H-1 of glucose) revealed nuclear Overhauser effects (NOEs) on the signals at δ 3.94 (H-3 of the aglycone), δ 4.39 (H-4 of rhamnose) and δ 4.32 (H-6 of glucose), respectively. Based on the above evidence, the sugar moiety of **7** was the same as **17**.[8] So, the structure of **7** was concluded to be thevetiogenin 3-*O*-β-gentiobiosyl-(1→4)-α-L-rhamnopyranoside.

Compound **4** was obtained as an amorphous powder and afforded a $[M+Na]^+$ ion peak at *m/z* 703 ($C_{35}H_{52}O_{13}Na$) in the positive-ion FAB-MS, 162 mass units less than that of **7**. Based on the ^{1}H- and ^{13}C-NMR spectra, **4** was determined to be a glycoside of thevetiogenin similar to **7**. Compound **4** exhibited two anomeric proton and carbon signals at δ 5.22 (d, J=8.0 Hz), δ 5.30 (d, J=1.0 Hz); and δ 99.6, δ 106.9, respectively.

Acid hydrolysis of **4** gave rhamnose and glucose, and the ratio of the sugars was 1 : 1. In the ROED spectra, irradiation at δ 5.30 (H-1 of rhamnose) and δ 5.22 (H-1 of glucose) revealed NOEs on the signals at δ 3.95 (H-3 of aglycone) and δ 4.37 (H-4 of rhamnose), respectively. Consequently, the structure of **4** was concluded to be

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thevetiogenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside.

Compound **15** was obtained as an amorphous powder and afforded a $[M+Na]^+$ ion peak at *m/z* 719 ($C_{36}H_{56}O_{13}Na$) in the positive-ion FAB-MS. Based on the ^1H- and ^{13}C-NMR signals, the aglycone was determined to be digitoxigenin[3a] and its spectra exhibited two anomeric proton and carbon signals at *δ* 5.27 (d, *J*=8.0 Hz) and *δ* 5.36 (d, *J*=2.0 Hz); and *δ* 99.5 and *δ* 105.7, respectively.

Acid hydrolysis of **15** gave acofriose and glucose, and the ratio of the sugars was 1 : 1. In the ROED spectra, irradiation at *δ* 5.36 (H-1 of acofriose) and *δ* 5.27 (H-1 of glucose) revealed NOEs on the signals at the *δ* 4.13 (H-3 of aglycone) and *δ* 4.47 (H-4 of acofriose), respectively. The sugar moiety of **15** was the same as **6**.[3a] Thus, the structure of **15** was established as digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-acofriopyranoside.

Compound **16** was obtained as an amorphous powder and afforded a $[M+Na]^+$ ion peak at *m/z* 761 ($C_{38}H_{58}O_{14}Na$) in the positive-ion FAB-MS. Based on the ^1H- and ^{13}C-NMR spectra, **16** was also determined to be a glycoside of digitoxigenin.[3a] It exhibited two anomeric proton and carbon signals at *δ* 5.32 (d, *J*=8.0 Hz) and *δ* 5.34 (d, *J*=4.0 Hz); and *δ* 94.1 and *δ* 104.9, respectively. Moreover the signal of one acetyl group was observed at *δ* 2.12 (3H, s) in the ^1H-NMR spectrum, and *δ* 20.8 and *δ* 170.4 in the ^{13}C-NMR spectrum.

Acid hydrolysis of **16** gave thevetose and glucose, and the ratio of sugars was 1 : 1. In the ROED spectra, irradiation at *δ* 5.34 (H-1 of thevetose) and *δ* 5.32 (H-1 of glucose) revealed NOEs on the signals at the *δ* 4.01 (H-3 of aglycone) and *δ* 3.90 (H-4 of thevetose), respectively. Since the ^1H-NMR signal of H-2 of the thevetosyl group was observed at *δ* 5.01 (dd, *J*=9.5, 4.0 Hz), a much lower magnetic field than that of **14**. So, the acetyl group was concluded to be located at the 2-OH of the thevetosyl group as thevetioside E.[3a] Thus, the structure of **16** was established as digitoxigenin 3-*O*-*β*-D-glucopyranosyl-(1→4)-2′-*O*-acetyl-α-L-thevetopyranoside.

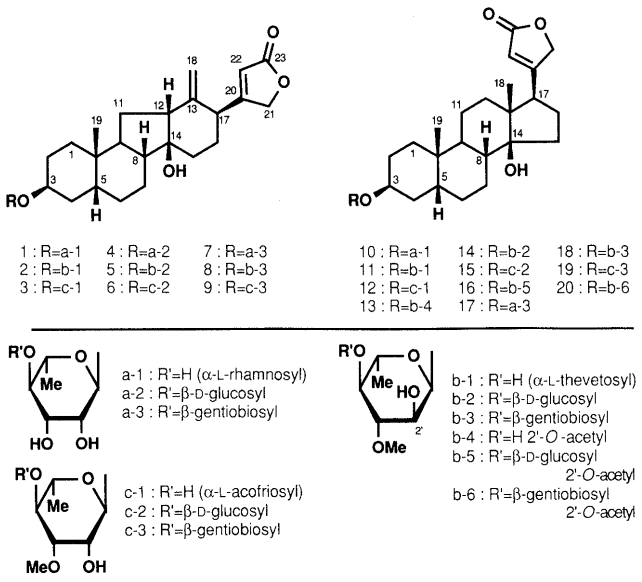

Fig. 1

Experimental

General Procedure Optical rotations were determined with a JASCO-360 digital polarimeter. FAB-MS spectra were recorded on a JEOL JMS-SX102 spectrometer. ^1H- and ^{13}C-NMR spectra were recorded on a JEOL JNM A-400 spectrometer (^1H-NMR; 400 MHz and ^{13}C-NMR; 100.40 MHz) in pyridine-d_5. Chemical shifts were given in *δ* values referred to internal tetramethylsilane (TMS). GC was carried out on a Hitachi G-3000 gas chromatograph. HPLC was carried out on a JASCO system 880 instrument.

Table 1. ^1H-NMR Spectral Data for **4**, **7**, **15** and **16**

Proton No.	**4**	**7**	**15**	**16**
Aglycone				
H-3	3.95 (1H, br s)	3.94[a]	4.13 (1H, br s)	4.01 (1H, br s)
9	2.64 (1H, m)	2.64 (1H, m)		
12	2.24 (1H, br t, *J*=9.5 Hz)	2.24 (1H, br t, *J*=9.5 Hz)		
17	3.47 (1H, br d, *J*=5.5 Hz)	3.47 (1H, br d, *J*=5.5 Hz)	2.81 (1H, dd, *J*=8.5, 6.0 Hz)	2.82 (1H, dd, *J*=9.5, 5.5 Hz)
18	5.10 (1H, br s)	5.11 (1H, br s)	1.04 (3H, s)	1.04 (3H, s)
	5.15 (1H, br s)	5.15 (1H, br s)	—	—
19	0.83 (3H, s)	0.83 (3H, s)	0.90 (3H, s)	0.92 (3H, s)
21	4.82 (2H, br s)	4.83 (2H, br s)	5.04 (1H, dd, *J*=18.5, 1.5 Hz)	5.04[a]
	—	—	5.31 (1H, dd, *J*=18.5, 1.5 Hz)	5.32[a]
22	6.24 (1H, d, *J*=2.0 Hz)	6.24 (1H, d, *J*=1.5 Hz)	6.14 (1H, br s)	6.14 (1H, br s)
Sugar				
1′	5.30 (1H, d, *J*=1.0 Hz)	5.28 (1H, br s)	5.36 (1H, d, *J*=2.0 Hz)	5.34 (1H, d, *J*=4.0 Hz)
2′	4.49 (1H, br s)	4.48 (1H, br s)	4.51 (1H, br s)	5.01 (1H, dd, *J*=9.5, 4.0 Hz)
3′	4.61 (1H, dd, *J*=9.5, 2.5 Hz)	4.59 (1H, dd, *J*=9.5, 3.5 Hz)	4.04 (1H, dd, *J*=9.5, 3.0 Hz)	4.09 (1H, t, *J*=9.5 Hz)
4′	4.37 (1H, t, *J*=9.5 Hz)	4.39 (1H, t, *J*=9.5 Hz)	4.47 (1H, t, *J*=9.5 Hz)	3.90 (1H, t, *J*=9.5 Hz)
6′	1.67 (3H, d, *J*=6.0 Hz)	1.78 (3H, d, *J*=6.0 Hz)	1.68 (3H, d, *J*=6.0 Hz)	1.60 (3H, d, *J*=6.0 Hz)
OMe	—	—	3.54 (3H, s)	3.83 (3H, s)
OMe	—	—	—	2.12 (3H, s)
1″	5.22 (1H, d, *J*=8.0 Hz)	5.18 (1H, d, *J*=7.5 Hz)	5.27 (1H, d, *J*=8.0 Hz)	5.32 (1H, d, *J*=8.0 Hz)
6″	4.35[a]	4.32 (1H, dd, *J*=12.0, 5.5 Hz)	4.34 (1H, dd, *J*=12.0, 4.5 Hz)	4.37 (1H, dd, *J*=11.5, 5.0 Hz)
	4.43 (1H, dd, *J*=12.0, 2.5 Hz)	4.71 (1H, dd, *J*=12.0, 1.5 Hz)	4.41 (1H, dd, *J*=12.0, 2.5 Hz)	4.54 (1H, br d, *J*=11.5 Hz)
1‴	—	5.08 (1H, d, *J*=7.5 Hz)	—	—

Measured at 400 MHz in pyridine-d_5 solution at 35°C. Chemical shifts are in *δ* values from internal tetramethylsilane (TMS) and are followed by multiplicities and *J* values (in Hz). *a*) Overlapping with other signals.

Table 2. ^{13}C-NMR Spectral Data of **4**, **7**, **15** and **16**

Carbon No.	4	7	15	16
C-1	32.4	32.5	30.1	29.9
2	27.0	27.0	26.9[a]	27.0[a]
3	72.4[a]	72.4[a]	72.4	72.9
4	30.8	30.7	31.1	31.0
5	36.8	36.8	37.2	37.0
6	28.2	28.2	27.2[a]	27.2[a]
7	20.9	20.9	22.0[b]	21.6[b]
8	50.4	50.4	42.0	42.0
9	38.7	38.7	35.9	35.8
10	35.4	35.4	35.6	35.6
11	21.9	21.9	21.6[b]	22.0[b]
12	50.6	50.5	40.0	39.9
13	147.7	147.7	50.2	50.2
14	79.9	79.9	84.7	84.6
15	31.9	31.9	33.3	33.2
16	25.4	25.3	27.4[a]	27.4[a]
17	44.5	44.5	51.5	51.5
18	110.5	110.5	16.3	16.2
19	22.9	22.9	24.1	24.2
20	173.4	173.4	175.9	175.9
21	73.1[b]	73.1[b]	73.8	73.7
22	116.1	116.1	117.7	117.7
23	174.3	174.4	174.5	174.5
1′	99.6	99.5	99.5	94.1
2′	72.5[a]	72.5[a]	68.5[c]	75.1
3′	72.9[b]	72.9[b]	82.7	82.0
4′	85.4	84.8	79.6	81.2
5′	68.3	68.2	68.3[c]	67.2
6′	18.4	18.7	18.7	18.4
OMe	—	—	56.7	61.1
1″	106.9	106.4	105.7	104.9
2″	76.5	75.3	76.1	75.6
3″	78.6[c]	78.4[c]	78.4[d]	78.4[c]
4″	71.6	71.6[d]	72.0	72.2
5″	78.5[c]	77.4	78.1[d]	78.3[c]
6″	62.8	70.2	63.0	63.2
1‴		105.5		
2‴		76.2		
3‴		78.5[c]		
4‴		71.7[d]		
5‴		78.5[c]		
6‴		63.8		
–OAc				20.8
				170.4

Measured at 100.40 MHz in pyridine-d_5 solution at 35 °C. Chemical shifts are in δ values from internal TMS. *a*—*d*) Interchangeable in each column.

Plant Material The leaves, wood and twigs of *Thevetia ahouai* (LINN.) A.DC., Apocynaceae, were collected in Costa Rica and the original plant was identified by Dr. Luis J. Poveda and Dr. Pablo E. Sanchez.

Extraction and Isolation The fresh leaves (490 g), wood (1 kg) and twigs (460 g) were extracted with 70% EtOH or Et_2O.

Isolation from the fresh leaves: The 70% EtOH extract was concentrated under reduced pressure, and the residue was partitioned between 80% aqueous MeOH and benzene–*n*-hexane (1 : 1). The 80% MeOH layer was concentrated under reduced pressure, and the residue was suspended in H_2O, and extracted with Et_2O. The Et_2O layer was concentrated, and the residue (1.3 g) was chromatographed on a silica-gel column with a $CHCl_3$–MeOH system and semi-preparative HPLC [ODS : CH_3CN–H_2O and MeOH–H_2O] to give **1** (11 mg), **2** (20 mg), **3** (13 mg). The H_2O layer was passed through a column of Mitsubishi Diaion HP-20, and the absorbed material was successively eluted with 50% aqueous MeOH, 70% aqueous MeOH and 100% MeOH. The eluates were concentrated under reduced pressure. The residue (1.0 g) from the 70% MeOH eluate was chromatographed in the same way as the Et_2O extract. The following compounds were isolated: **1** (2 mg), **4** (1 mg), **5** (2 mg), **6** (5 mg), **7** (3 mg), **8** (3 mg), **9** (12 mg), **17** (2 mg), **19** (7 mg). The residue (0.7 g) from the MeOH eluate was chromatographed as described above to give **2** (2 mg), **3** (4 mg), **10** (9 mg), **20** (3 mg).

Isolation from the fresh wood: This was treated in the same way as the fresh leaves. The following compounds were obtained: **2** (6 mg), **3** (8 mg), **11** (11 mg), **12** (32 mg), **13** (21 mg), **16** (2 mg).

Isolation from the fresh twigs: This was treated in the same way as the fresh leaves. The following compounds were obtained: **14** (7 mg), **15** (3 mg), **18** (5 mg), **19** (4 mg), **20** (8 mg).

Known compounds were identified by comparison of the spectral data NMR, MS and specific optical rotation with the literature values, and the structures were checked with 2D-NMR.

Compound 4 Amorphous powder. $[\alpha]_D^{21}$ +37.7° (*c*=0.15, MeOH). FAB-MS *m/z*: 703 $[M+Na]^+$.

Compound 7 Amorphous powder. $[\alpha]_D^{20}$ +5.6° (*c*=0.35, MeOH). FAB-MS *m/z*: 865 $[M+Na]^+$.

Compound 15 Amorphous powder. $[\alpha]_D^{23}$ −29.7° (*c*=0.32, MeOH). FAB-MS *m/z*: 719 $[M+Na]^+$.

Compound 16 Amorphous powder. $[\alpha]_D^{24}$ −20.6° (*c*=0.26, MeOH). FAB-MS *m/z*: 761 $[M+Na]^+$.

The ^{1}H- and ^{13}C-NMR chemical shifts of **4**, **7**, **15** and **16** are shown in Tables 1 and 2, respectively.

Acid Hydrolysis of 4, 7, 15 and 16 Compounds **4**, **7**, **15** or **16** (*ca.* 0.1 mg) were dissolved in dioxane and 5% H_2SO_4 (three drops each) and the solution was heated at 100 °C for 1 h. After hydrolysis, the solution was passed through a column of Amberlite IRA-60E, and the eluate was concentrated to give a residue, to which $NaBH_4$ (*ca.* 1 mg) was added and left stand for 1 h at r.t. The reaction mixture was deionized using an Amberlite IR-120B column and the filtrate was concentrated to dryness. Boric acid was removed by co-distillation with MeOH and the residue was acetylated with acetic anhydride and pyridine (three drops each) at r.t. for overnight. The reagents were evaporated *in vacuo*, and the residue was subjected to GC analysis. The GC conditions were follows: column, Spelco SP-2380 capillary column (0.25 mm × 30 m), injection port temp. 250 °C, detector 250 °C, carrier gas; N_2 3.0 ml/min. Retention times, t_R (min), of the acetates were as follows: Column temp. 217 °C, acofritol acetate 10.6, thevetitol acetate 11.2, and rhamnitol acetate 11.0. Column temp. 250 °C, glucitol acetate 13.5.

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