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Iridoids from the Roots of *Thevetia peruviana*

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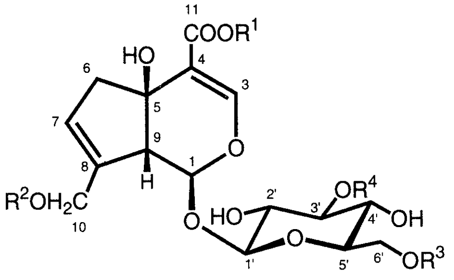
10-*O*-*β*-D-Glucopyranosyltheviridoside and 3′-*O*-*β*-D-glucopyranosyltheviridoside were obtained from the roots of *Thevetia peruviana* along with the major iridoids, theviridoside and theveside, and minor ones, recently obtained from the leaves, 10-*O*-fructofuranosyltheviridoside and 6′-*O*-glucopyranosyltheviridoside.

Key words *Thevetia peruviana*; 10-*O*-glucosyltheviridoside; 3′-*O*-glucosyltheviridoside; Apocynaceae; iridoid

Thevetia peruviana (PERS.) K. SCHUM. (Apocynaceae) is a short tree indigenous to the tropical region of the American Continent. In reports on the constituents of the leaves, we have described cardenolides$^{1)}$ and iridoids.$^{2)}$ This paper deals with two new glucosides of theviridoside from the roots.

When the roots were percolated with MeOH and the extract was fractionated in the usual manner, the major iridoids theveside (**1**)$^{3)}$ and theviridoside (**2**)$^{4)}$ were isolated from the polar fraction. Four compounds (**3**—**6**), showing the same blue staining as **1** and **2** with H_2SO_4 reagent, were isolated, along with **1** and **2**, by successive chromatographies on reversed-phase and normal-phase columns and finally by preparative HPLC. Compounds **3** and **4** were identified as 10-*O*-*β*-D-fructofuranosyltheviridoside and 6′-*O*-*β*-D-glucopyranosyltheviridoside, respectively, by comparison with authentic samples obtained from the leaves.$^{2)}$



1 : $R^1=R^2=R^3=R^4=H$
2 : $R^1=CH_3$, $R^2=R^3=R^4=H$
3 : $R^1=CH_3$, R^2=β-D-fructofuranosyl, $R^3=R^4=H$
4 : $R^1=CH_3$, $R^2=R^4=H$, R^3=β-D-glucopyranosyl
5 : $R^1=CH_3$, R^2=β-D-glucopyranosyl, $R^3=R^4=H$
6 : $R^1=CH_3$, $R^2=R^3=H$, R^4=β-D-glucopyranosyl

Chart 1

TABLE I. ^1H- and ^{13}C-NMR Data for **2**, **5** and **6** (δ ppm from TMS in C_5D_5N, J=Hz in Parentheses)

No. of C or H	**5**			**6**			**2**
	C	H	*a*)	C	H	*a*)	C
1	98.4	5.84 (d, 7)	C-5, 1′	98.9	5.79 (d, 7)$^{d)}$	C-3, 5, 1′	99.1
3	152.5	7.62 (s)	C-1, 4, 11	152.4	7.66 (s)	C-1, 4, 5, 11	152.2
4	115.0			115.3			115.2
5	76.0			76.4			76.5
6	47.3	2.90 (br d, 17)	C-8	47.5	2.94 (br d, 17)		47.5
		3.16 (br d, 17)	C-5, 8		3.21 (br d, 17)		
7	127.3	5.92 (br s)	C-5	125.2	5.99 (br s)	C-5, 9	125.2
8	139.0			143.2			143.3
9	56.8	3.55 (d, 7)	C-1, 5, 7, 8	57.1	3.47 (d, 7)	C-1, 5, 7, 8	57.2
10	68.2	4.94 (br d, 13)	C-7, 8	60.8	4.74 (br d, 14)	C-7, 8	60.9
		4.56 (br d, 13)			4.50 (br d, 14)	C-7, 8	
11	167.0			167.1			167.1
OMe	50.8	3.59 (s)	C-11	50.9	3.62 (s)	C-11	50.9
1′	100.7	5.35 (d, 8)	C-1	100.4	5.33 (d, 8)$^{d,e)}$	C-1	100.9
2′	74.8	4.06 (t, 8)	C-1′, 3′	73.5	4.05 (t, 8)	C-1′, 3′	74.8
3′	78.3$^{b)}$	4.21 (t, 8)		88.1	4.24 (t, 8)$^{f)}$	C-2′, 1″	78.8$^{b)}$
4′	71.6$^{c)}$	4.24 (t, 8)		69.6	4.05 (t, 8)		71.4
5′	78.3$^{b)}$	3.98 (m)		78.4	3.89 (m)$^{e)}$		78.3$^{b)}$
6′	62.7	4.49 (dd, 12, 1)	C-4′	62.1	4.38 (dd, 12, 2)	C-4′	62.5
		4.30 (dd, 12, 5)			4.19 (dd, 12, 5)		
1″	104.7	4.88 (d, 8)	C-10, 5″	105.8	5.30 (d, 8)$^{f,g)}$	C-3′	
2″	75.2	4.03 (t, 8)	C-1″, 3″	75.6	4.05 (t, 8)		
3″	78.4$^{b)}$	4.19 (t, 8)		78.2	4.22 (t, 8)		
4″	71.5$^{c)}$	4.17 (t, 8)	C-3″	71.6	4.17 (t, 8)		
5″	78.8	3.85 (m)		78.6	3.99 (m)$^{g)}$		
6″	62.7	4.49 (dd, 12, 1)		62.5	4.51 (dd, 12, 2)		
		4.30 (dd, 12, 5)	C-4″		4.29 (dd, 12, 5)		

a) Correlated carbon signals from the corresponding proton signal in the HMBC spectra. *b*, *c*) Signal assignments may be interchangeable in each column. *d*—*f* or *g*) A cross peak was observed between these signals in the 2D-NOESY spectra.

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Compounds **5** and **6** showed the same *Rf* value as that of **4** on TLC, suggesting that they have similar molecular formulae and structures to **4**. Based on the ^{1}H- and ^{13}C-NMR signals, **5** seemed to include the structure of **2**. Two anomeric proton signals were observed at δ 4.88 (d, $J=8$ Hz) and 5.35 (d, $J=8$ Hz), of which the latter was due to the glucose unit in **2**. The additional sugar was assignable as glucose based on the ^{13}C-signals. In the aglycone moiety, the C-10 signal was observed at lower field (+7.3 ppm) and the C-8 signal at upper field (−4.3 ppm) in comparison with those of **2**, indicating that the glycosidic linkage is at C-10. In the HMBC spectrum of **5**, three-bond correlations were observed between H-1″/C-10 and H-1′/C-1. The structure of 5 was therefore determined to be the 10-*O*-*β*-D-glucopyranoside of **2**.

Compound **6** was observed to contain the structure of **2** in the molecule, together with two glucose units as in **4** and **5**, based on the NMR spectra. The linkage position of the second glucose unit was assigned to be 3′-OH of **2**, based on the glycosylation shift at C-3′ (+9.3 ppm), nuclear Overhauser effect (NOE) between H-1″/H-3′ and the cross peak between H-1″/C-3′ in the heteronuclear multiple bond conectivity (HMBC) spectrum. Thus, **6** was characterized as the 3′-*O*-*β*-D-glucopyranoside of **2**.

While **3** and **4** had previously been obtained from the leaves frozen at −20 °C after harvest, neither **5** nor **6** was observed. The possibility that 11-demethyl glycosides corresponding to **3**—**6** are present in the fraction containing **1** can not be excluded.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS). FAB-MS were recorded on a JEOL D-300 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column chromatography and TLC, the following solvent systems were applied: $CHCl_3$–MeOH–H_2O (bottom layer, solvent 1), EtOAc–MeOH–H_2O (top layer, solvent 2).

Extraction and Isolation of Iridoids *Thevetia peruviana* (Pers.) K. Schum. was cultivated in the greenhouse of Nagasaki University for 3 years from seeds which had been collected in Taiwan. Air-dried roots (750 g) were powdered and percolated with MeOH and the MeOH percolate was concentrated *in vacuo* to 1 l. To the concentrated MeOH solution, H_2O (1 l) was added. The precipitate was filtered off and the filtrate was extracted with benzene. The H_2O layer was then concentrated *in vacuo* and passed through an MCI-gel column (polystyrene, CHP-20P, Mitsubishi Chem. Ind.). The column was eluted first with H_2O, and then with H_2O–MeOH mixture with increasing MeOH. The eluate with 25% MeOH (ext. 1.62 g) was then chromatographed on an octadecylsilica (ODS) column (YMC-gel) with MeCN–H_2O mixture (MeCN: 0—20%). The 1st fraction contained **1** (0.1 g). The 2nd fraction, showing a blue spot on TLC with an intermediate *Rf* value between those of **1** and **2**, was subjected to silica gel column chromatography with solvent 1 and then preparative HPLC on an ODS column with 10% MeCN to give **3** (6 mg), **4** (60 mg), **5** (6 mg), **6** (28 mg) and **2**. The fraction obtained after elution of **3**—**6** on an MCI-gel column (50% MeOH elution) afforded **2** (2 g in total).

10-*O*-*β*-D-Glucopyranosyltheviridoside (5)

Solid, $[\alpha]_D^{26}$ −8.2° ($c=0.22$, MeOH), FAB-MS *m/z*: 589.1746 (Calcd for $C_{23}H_{34}O_{16}+Na$: 589.1744). ^{1}H- and ^{13}C-NMR: see Table I.

3′-*O*-*β*-D-Glucopyranosyltheviridoside (6)

Solid, $[\alpha]_D^{27}$ −19.1° ($c=1.40$, MeOH), FAB-MS *m/z*: 589.1743 (Calcd for $C_{23}H_{34}O_{16}+Na$; 589.1744). ^{1}H- and ^{13}C-NMR: see Table I.

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