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DINORMONOTERPENOIDS AND THEIR APIOSYLGLUCOSIDES FROM *THEVETIA PERUVIANA*

FUMIKO ABE, RONG-FUH CHEN and TATSUO YAMAUCHI

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka 814-80, Japan

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Key Word Index—*Thevetia peruviana*; Apocynaceae; polyhydroxydinormonoterpenoid; dinormonoterpenoid apiosylglucoside.

Abstract—Polyhydroxy-dinormonoterpenoids and their apiosylglucosides were obtained from the polar fraction of the leaves of *Thevetia peruviana*. Copyright © 1996 Elsevier Science Ltd

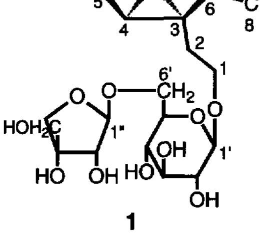
INTRODUCTION

In the course of our studies on iridoids and related compounds from Apocynaceae plants, we described the isolation of four iridoid biosides composed of theviridoside and D-fructose or D-glucose, from the leaves and roots of *Thevetia peruviana* (Pers.) K. Schum. [1, 2]. In further investigations of the polar fraction, the presence of polyhydroxy-dinormonoterpenoids and their biosides was revealed.

RESULTS AND DISCUSSION

When the MeOH percolate of the air-dried leaves was fractionated on a polystyrene column, and the 25% MeOH eluate was further fractionated using reversed-phase and normal-phase columns, five compounds (**1**–**5**), showing almost the same polarity as theviridoside (**6**), were isolated.

The FAB-mass spectrum of **1** afforded a $[M + Na]^+$ peak at m/z 477.1948, suggesting the molecular formula, $C_{19}H_{34}O_{12}$. Since two anomeric proton signals were observed at δ 4.79 (*d*, $J = 8$ Hz) and 5.75 (*d*, $J = 2$ Hz), **1** seemed to be a bioside, and the sugar moiety was assignable to β-D-apiosyl(1→6)-β-D-glucoside, based on a consideration of the ^1H and ^{13}C NMR spectra, including ^1H-^1H and ^1H-^{13}C COSY and HMBC between H-1″(apiose) and C-6′(glucose) and a comparison with the spectra of a known apiosyl(1→6)-glucoside [3]. The aglycone moiety was considered to be composed of eight carbons. In the ^1H NMR spectrum, the presence of two methyl groups (δ 1.39, 1.40) on a quarternary carbon and one methyl group (δ 1.62, *d*, $J = 6$ Hz) linked to a secondary carbon having one methine proton (δ 3.25, *q*, $J = 6$ Hz), and one hydroxymethylene group (δ 3.84, 4.18) adjacent to a methylene group (δ 2.08, 2.35) were suggested. In the ^{13}C NMR spectrum, two oxygenated



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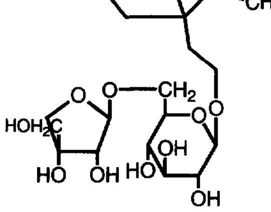

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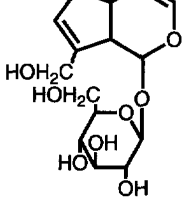

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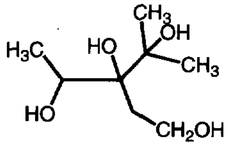

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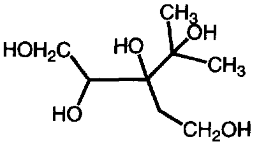

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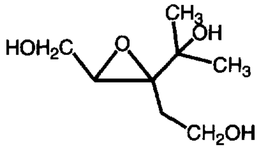

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quaternary carbon signals were observed at δ 66.0 and 70.6, along with one tertiary carbon signal (δ 58.8) and one secondary carbon (δ 66.0) signal. One of the quaternary carbons seemed to be part of a hydroxyisopropyl group. The degree of unsaturation of **1** was calculated as three, two were due to sugar rings, and the presence of one epoxide ring was suggested. The location of the epoxide was assigned to C-3 (δ 66.0) and C-4 (δ 58.8), based on the 2-bond and 3-bond correlations of the methyl protons of the hydroxyisopropyl group, the methylene protons at C-2 or the methyl protons at C-5, to the epoxide carbons. The presence of NOEs between the methyl protons of the hydroxyisopropyl group and H-5, and H-4 and C-2 methylene protons showed the relative configurations of the hydroxyisopropyl group and the methyl group of C-5 to be *cis*.

The molecular formula of **2**, $C_{19}H_{36}O_{12}$, was 2 mu higher than that of **1**. The component sugar was assigned as glucose, based on the ^{1}H and ^{13}C NMR spectra. In the aglycone moiety, two quaternary carbon signals (δ 75.4, 77.2) were observed in the carbinol carbon region as in **1**, while a 3H triplet signal (δ 1.10, $J = 6$ Hz), instead of a 3H doublet signal due to the terminal methyl protons of C-5 in **1**, suggested the presence of methylene protons at C-4 and a tertiary hydroxyl group at C-3. The array of each carbon was confirmed by HMBC of the aglycone moiety, as well as the glycosidic linkage of the primary carbinol at C-1 (δ 67.6).

Compounds **3–5** were free dinormonoterpenoids and the presence of the hydroxyethyl moiety composed of C-1 and C-2 was confirmed by a triplet (δ 58.2–58.9) and a doublet (δ 35.6–36.4) of ^{13}C signals and the corresponding ^{1}H signals in the NMR spectra. FAB mass spectrum of **3** suggested a molecular formula of $C_8H_{18}O_4$. Two 3H singlet signals at δ 1.47–1.66 were assignable to the hydroxyisopropyl group as in **1** and **2**. Since terminal methyl protons appeared as a doublet signal ($J = 6$ Hz), and the proton linked to the adjacent carbon was observed as a quartet signal at δ 4.40 ($J = 6$ Hz), **3** was characterized as a 1,3,4,6-tetrahydroxyoctane having the same carbon array as in the aglycones of **1** and **2**.

The molecular formula of **4**, $C_8H_{18}O_5$, contained one oxygen more than **3**, based on the mass spectra. On comparison of the ^{1}H and ^{13}C NMR spectra, C-5 in **4** showed a significant lowerfield shift from δ 19.2 in **3** to δ 66.2. C-4 was also shifted to lowerfield from δ 71.5 to δ 76.8. The structure of **4** was thus assigned to be a 1,3,4,5,6-tetrahydroxyoctane. In **5**, C-5 was observed as a triplet signal at δ 61.0 as in **4**, while C-3 and C-4 were observed at δ 67.4 and 66.0, respectively, and were higherfield in comparison with those of **3** and **4**. Since FAB-mass spectra of **5** suggested a molecular formula of $C_8H_{16}O_4$, **5** was determined to be the C-3,4 epoxide form of **4**.

In a previous study, we described the isolation of cerbidols [4], cyclopentanonormonoterpenoids, from the leaves of *Cerbera manghas*, a South-east Asian plant closely related to the Central American genus, *Thevetia*. Both genera are known to contain the same

Table 1. ^{1}H NMR spectral data for compounds **1–4** and **5** [δ (ppm) in pyridine-d_5 (400 MHz)]

H	1	2	3	4	5
1	3.84 (*m*) 4.18 (*m*)	4.18 (*m*) 4.62 (*m*)	4.22 (2H, *m*)	4.32 (2H, *m*)	3.79 (2H, *m*)
2	2.08 (*qui*, 7)*‡ 2.35 (*qui*, 7)*	2.21 (*qui*, 7) 2.38 (*qui*, 7)	2.22 (2H, *m*)	2.34 (*dt*, 15, 6) 2.43 (*dt*, 15, 6)	2.16 (*dt*, 15, 7) 2.40 (*dt*, 15,7)
4	3.25 (*q*, 6)	1.75 (*sex*, 6) 1.88 (*sex*, 6)	4.40 (*q*, 6)	4.50 (*dd*, 7, 4)	3.80 (*dd*, 6, 3)
5	1.62 (*d*, 6)†	1.10 (*t*, 6)	1.58 (*d*, 6)	4.39 (*dd*, 13, 7) 4.51 (*dd*, 13, 4)	4.53 (*dd*, 13, 3) 4.70 (*dd*, 13, 6)
7, 8	1.39 (*s*)†‡ 1.40 (*s*)	1.41 (*s*) 1.44 (*s*)	1.54 (*s*) 1.55 (*s*)	1.59 (*s*) 1.66 (*s*)	1.47 (*s*) 1.50 (*s*)
1′	4.79 (*d*, 8)	4.84 (*d*, 7)			
2′	3.97 (*t*, 8)	3.99 (*dd*, 7, 8)			
3′	4.18 (*t*, 8)	4.16 (*t*, 8)			
4′	4.02 (*t*, 8)	4.01 (*t*, 8)			
5′	4.03 (*m*)	4.03 (*m*)			
6′	4.14 (*dd*, 12, 5) 4.68 (*dd*, 12, 1)	4.17 (*dd*, 12, 5) 4.68 (*dd*, 12, 2)			
1″	5.75 (*d*, 2)	5.77 (*d*, 2)			
2″	4.69 (*d*, 2)	4.69 (*d*, 2)			
4″	4.32 (*d*, 9) 4.55 (*d*, 9)	4.32 (*d*, 9) 4.56 (*d*, 9)			
5″	4.12 (*d*, 11) 4.15 (*d*, 11)	4.12 (*d*, 10) 4.15 (*d*, 10)			

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

*,†,‡NOE was observed between these signals in the DIF-NOE spectrum.

principle cardenolides and iridoids. In this study, however, only acyclic dinormonoterpenoids but no cyclopentano-compounds were obtained.

EXPERIMENTAL

General. ^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz in pyridine-d_5 and TMS as int. standard. TLC and silica gel CC: solvent systems used, 1: $CHCl_3$–MeOH–H_2O (bottom layer); 2: EtOAc–MeOH–H_2O (upper layer).

Plant materials. The seeds of *T. peruviana* (Perrs.) K. Schum., collected in Taiwan, were sown and cultivated in a greenhouse of Nagasaki University at Nomo for 3 years [2].

Extraction and isolation. The leaves were collected in July, 1992, and air-dried in the shade. The dried leaves (1.1 kg) were powdered and percolated with MeOH. The percolate was concd *in vacuo*, passed through an MCI-gel column (polystyrene column) and eluted with MeOH–H_2O. The eluate with 25% MeOH (1.08 g) was then chromatographed on a YMC-gel column with MeCN–H_2O containing an increasing MeCN concentration. Compounds **1–5**, showing dark-yellowish staining on TLC with dil. H_2SO_4 after spraying and heating, with a similar R_f value as theviridoside, were obtained by further chromatography with solvents 1 and 2 on a silica gel column, and HPLC with 5% MeCN. **1**: 55 mg, **2**: 11 mg, **3**: 8 mg, **4**: 7 mg, **5**: 26 mg.

Compound **1**. Solid, $[\alpha]_D^{23}$ $-$81.7° (MeOH; *c* 2.75), FAB-MS *m/z*: 477.1948, $C_{19}H_{34}O_{12}Na$ requires 477.1948.

Compound **2**. Solid, $[\alpha]_D^{20}$ $-$55.1° (MeOH; *c* 0.54), FAB-MS *m/z*: 479.2104, $C_{19}H_{36}O_{12}Na$ requires 479.2105.

Compound **3**. Solid, $[\alpha]_D^{28}$ +5.78° (MeOH; *c* 0.38), FAB-MS *m/z*: 201.1103, $C_8H_{18}O_4Na$ requires 201.1102.

Compound **4**. Solid, $[\alpha]_D^{27}$ +1.09° (MeOH; *c* 0.37), FAB-MS *m/z*: 217.1053, $C_8H_{18}O_5Na$ requires 217.1051.

Table 2. ^{13}C NMR spectral data for compounds **1–4** and **5** [δ (ppm) in pyridine-d_5 (100 MHz)]

C	**1**	**2**	**3**	**4**	**5**
1	66.0	67.6	58.9	59.0	58.2
2	33.2	34.7	35.6	36.3	36.4
3	66.0	77.2	78.4	78.6	67.4
4	58.8	28.7	71.5	76.8	66.0
5	14.4	9.4	19.2	66.2	61.0
6	70.6	75.4	76.2	76.0	70.5
7, 8	26.6	25.8	26.1	26.2	27.0
	28.6	25.8	26.5	26.4	28.0
1′	104.5	104.6			
2′	75.0	75.1			
3′	78.5	78.6			
4′	71.7	71.8			
5′	77.1	77.7			
6′	68.8	68.7			
1″	111.1	111.0			
2″	77.8	77.7			
3″	80.4	80.4			
4″	75.0	75.0			
5″	65.6	65.7			

Compound **5**. Solid, $[\alpha]_D^{28}$ +1.76° (MeOH; *c* 1.30), FAB-MS *m/z*: 199.0948, $C_8H_{16}O_4Na$ requires 199.0946.

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