Food Chemistry 115 (2009) 61–65

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Sweet and bitter constituents of *Wilbrandia* species

Masamitsu Ochi [a], Koichi Moriyama [a], Kiyokazu Ohmae [a], Yoshiyasu Fukuyama [b], Ken-ichi Nihei [c], Isao Kubo [c,*]

[a] Faculty of Science, Kochi University, Akebono-cho, Kochi 780-8520, Japan
[b] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan
[c] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720-3114, USA

ARTICLE INFO

Article history:
Received 23 July 2008
Received in revised form 14 October 2008
Accepted 19 November 2008

Keywords:
Wilbrandiside A
Wilbrandiside B
Cucurbitane triterpenoid
Saponin
Cucurbitaceae
Sweet-taste compound

ABSTRACT

Two novel cucurbitane glycosides, wilbrandisides A and B were isolated as sweet-taste substances from the root of *Wilbrandia* species (*Cucurbitaceae*) along with seven known cucurbitane glycosides. Their structures were determined by spectroscopic means, including two-dimensional NMR experiments. Their sweet-taste properties were evaluated by a human sensory panel test. Consequently, wilbrandiside A was shown to be 28 times sweeter than sucrose and was the compound having the most potent sweet taste of all the cucurbitane glycosides isolated from this plant.

1. Introduction

The root called "cabeça-de-negro", is a *Wilbrandia* species occurring in northeastern Brazil and locally known as "cabeça-de-negro" (black head in English) because the shape of this root looks like a head with black hair. This extremely bitter tasting root is used in folk medicine for the treatment of diarrhoea, rheumatism and fever (Braga, 1960). The root of cabeça-de-negro tastes extremely bitter and the isolation of cucurbitacin related compounds was previously reported (Matos, Machado, Craveiro, Matos, & Braz-Filho, 1991).

Information on the surrounding environment of living plants often provides hints in selecting a plant collection. However, there is usually no information available when the dried plants are sold at market places, the bitter taste is often an initial clue on the selection of plants for further study since many antifeedants characterised from plants are bitter tasting (Kubo, 1993). For example, ajugarins (Kubo, Lee, Balogh-Nair, Nakanishi, & Chapya, 1976), azadirachtin (Butterworth & Morgan, 1968) and zumsin (Nihei, Hanke, Asaka, Matsumoto, & Kubo, 2002) are all bitter tasting. Interestingly, in our preliminary leaf-disc assay using two Lepidoptera larvae, the pink bollworm *Pectinophora gossypiella* and cotton budworm *Heliothis virescens* (Zhang & Kubo, 1992), the EtOH extract of cabeça-de-negro did not show any antifeedant activity against these insects up to 5 mg/disc, but the feeding was slightly stimulated. The observation of this adverse effect prompted us to test the individual purified compounds and purification was achieved. The aim of this paper is to report the isolation of eight cucurbitacin related bitter principles, emphasising two novel glycosides. Notably, these two novel glycosides showed sweet taste.

2. Experimental

2.1. General experimental procedures

IR spectra were recorded on a JASCO FTIR-5300 spectrometer using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 600 or 400 instrument (TMS as internal reference). HRMS-FAB was measured in the positive mode on a JEOL AX-500 spectrometer using glycerol as the matrix. Optical rotation was recorded on a JASCO DIP-1000 digital polarimeter. Middle pressure liquid chromatography (MPLC) was performed using Merck Lobar column (RP-8). Preparative HPLC was performed on a Waters 6000 A pump with a UV detector and a Cosmosil 5C18-AR column (5 μm, 10 mm × 250 mm).

2.2. Plant material

The fresh root of *Wilbrandia* species (*Cucurbitaceae*), locally known as "cabeça-de-negro" (black head in English), was purchased from an open market in Juazeiro do Norte, Ceará, Brazil.

* Corresponding author. Tel.: +1 510 643 6303; fax: +1 510 643 0215.
E-mail address: ikubo@berkeley.edu (I. Kubo).

0308-8146/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.11.062

The root was immediately cut into small pieces near the site of the purchase and air dried in the shade. The dried powder was kept at ambient temperature until use. A voucher specimen was deposited to Dr. J.M. Pines, Goeldi Museum, Belém, Pará, Brazil.

2.3. Extraction and isolation

The dried powdered root (500 g) was extracted with EtOH at ambient temperature. The solvent was evaporated *in vacuo*, and then, the resulting residue (65 g) was partitioned between H_2O and organic solvents, *n*-hexane, EtOAc, and *n*-BuOH in this order. The *n*-BuOH fraction was subjected to silica gel chromatography eluted with 40% MeOH/$CHCl_3$. The sweet-taste fraction obtained was subjected to silica gel chromatography eluted with 30% MeOH/$CHCl_3$ containing 5% H_2O, and then, RP-MPLC eluted with 30–40% MeOH/H_2O to give **1** (55 mg), **2** (66 mg), **3** (31 mg), and **4** (42 mg) as an amorphous solid. The EtOAc fraction was subjected to silica gel chromatography eluted with 30–40% MeOH/$CHCl_3$ and the further purification by preparative HPLC gave **5** (27 mg), **6** (54 mg), **7** (20 mg), and **8** (28 mg) as an amorphous solid.

2.4. Wilbrandiside A (*1*)

$[\alpha]_D^{27}$ −69° (*c* 0.20, EtOH); HRFABMS, 983.5168 ([M+Na]$^+$, calculated 983.5192 for $C_{48}H_{80}O_{19}Na$); IR (KBr) ν_{max}: 3400, 1685, 1641 cm^{-1}; ^{1}H and ^{13}C NMR assignments are shown in Tables 1 and 2.

Table 1
^{1}H and ^{13}C NMR assignments for the aglycons of **1** and **2** in pyridine-d_5.

Position	**1**		**2**	
	δ_H (mult. *J* in Hz)	δ_C (mult.)	δ_H (mult. *J* in Hz)	δ_C (mult.)
1	1.61 (m)	22.18 (t)	1.60 (m)	22.59 (t)
	1.98 (m)		1.85 (m)	
2	1.89 (m)	28.59 (t)	1.96 (m)	28.59 (t)
	2.41 (m)		2.58 (m)	
3	3.70 (s)	86.59 (d)	3.70 (s)	86.23 (d)
4		41.96 (s)		40.96 (s)
5		141.30 (s)		142.12 (s)
6	5.49 (d, 5.9)	118.40 (d)	5.41 (d, 5.4)	118.40 (d)
7	1.74 (dd, 11.8, 5.9)	24.09 (t)	1.64 (m)	23.45 (t)
	2.13 (dd, 11.8, 8.1)		2.14 (m)	
8	1.77 (d, 8.1)	43.92 (d)	1.64 (m)	37.42 (d)
9		49.05 (s)		33.61 (s)
10	2.41 (m)	35.92 (d)	2.21 (m)	37.42 (d)
11		213.75 (s)	1.63 (m)	31.36 (t)
			1.33 (m)	
12	1.46 (m)	48.76 (t)	1.46 (m)	29.66 (t)
	1.63 (m)		1.63 (m)	
13		48.97 (s)		45.55 (s)
14		49.67 (s)		47.95 (s)
15	1.23 (dd, 10.5, 10.0)	34.48 (t)	1.06 (dd, 10.0, 9.5)	33.61 (t)
	1.70 (d, 10.0)		1.15 (d, 10.0)	
16	1.28 (d, 10.5)	28.10 (t)	1.49 (m)	26.32 (t)
	1.98 (m)		2.19 (d, 9.5)	
17	1.71 (m)	49.99 (d)	1.79 (m)	46.68 (d)
18	0.69 (s)	16.96 (q)	0.84 (s)	14.48 (q)
19	0.85 (s)	20.26 (q)	0.85 (s)	27.06 (q)
20	1.40 (m)	36.21 (d)	2.16 (m)	42.16 (d)
21	0.90 (d, 6.4)	18.63 (q)	1.29 (d, 6.6)	12.20 (q)
22	1.07 (m)	34.38 (t)	4.55 (m)	68.66 (t)
	1.67 (m)			
23	1.51 (m)	28.86 (t)	1.82 (m)	31.99 (t)
	1.71 (m)		2.00 (m)	
24	4.00 (m)	75.56 (d)	4.65 (d, 10.0)	70.92 (d)
25		80.51 (s)		79.50 (s)
26	1.52 (s)	22.57 (q)	1.59 (s)	21.83 (q)
27	1.52 (s)	22.90 (q)	1.61 (s)	21.83 (q)
28	1.03 (s)	28.28 (q)	1.03 (s)	27.34 (q)
29	1.49 (s)	25.77 (q)	1.49 (s)	24.76 (q)
30	0.94 (s)	18.17 (q)	0.77 (s)	16.96 (q)

Table 2
^{1}H and ^{13}C NMR assignments for the glycosides of **1** and **2** in pyridine-d_5.

Position	**1**		**2**	
	δ_H (mult. *J* in Hz)	δ_C (mult.)	δ_H (mult. *J* in Hz)	δ_C (mult.)
Glc				
1'	4.79 (d, 7.6)	106.97 (d)	4.73 (d, 8.2)	105.89 (d)
2'	3.89 (dd, 8.6, 7.6)	75.37 (d)	3.91 (dd, 8.2, 8.0)	74.26 (d)
3'	4.11 (dd, 9.0, 8.6)	78.63 (d)	4.09 (dd, 8.0, 7.1)	77.48 (d)
4'	4.00 (dd, 9.0, 7.8)	71.76 (d)	3.98 (dd, 8.1, 7.1)	70.58 (d)
5'	4.07 (m)	77.34 (d)	4.08 (m)	76.31 (d)
6'	4.29 (d, 11.2)	70.47 (t)	4.30 (d, 9.3)	70.47 (t)
	4.81 (d, 11.2)		4.83 (d, 9.3)	
Glc				
1''	5.13 (d, 7.8)	105.50 (d)	5.19 (d, 7.3)	104.20 (d)
2''	4.05 (dd, 9.5, 7.8)	75.25 (d)	4.06 (dd, 7.3, 7.1)	74.12 (d)
3''	4.22 (dd, 9.5, 8.8)	78.46 (d)	4.25 (dd, 9.5, 7.1)	77.35 (d)
4''	4.20 (dd, 8.8, 7.6)	71.72 (d)	4.25 (m)	70.65 (d)
5''	3.94 (m)	78.52 (d)	3.98 (m)	77.43 (d)
6''	4.38 (d, 11.5)	62.77 (t)	4.33 (dd, 11.7, 2.4)	61.68 (t)
	4.51 (d, 11.5)		4.47 (dd, 11.7, 5.8)	
Glc				
1'''	5.22 (d, 7.8)	97.57 (d)	5.21 (d, 7.3)	96.54 (d)
2'''	4.01 (dd, 7.8, 8.1)	75.70 (d)	4.06 (dd, 7.3, 8.1)	74.42 (d)
3'''	4.23 (m)	78.96 (d)	4.13 (dd, 8.1, 7.7)	77.67 (d)
4'''	4.19 (dd, 8.8, 7.6)	71.72 (d)	4.16 (dd, 8.8, 7.7)	70.51 (d)
5'''	3.94 (m)	78.46 (d)	4.13 (m)	77.32 (d)
6'''	4.19 (d, 13.7)	62.77 (t)	4.28 (m)	61.68 (t)
	4.49 (d, 13.7)		4.39 (m)	

2.5. Wilbrandiside B (*2*)

$[\alpha]_D^{27}$ −62° (c 0.22, EtOH); HRFABMS, 985.5344 ([M+Na]$^+$ calculated 985.5348 for $C_{48}H_{82}O_{19}Na$); IR (KBr) ν_{max}: 3395, 1640 cm^{-1}; ^{1}H and ^{13}C NMR assignments are shown in Tables 1 and 2.

2.6. Identification of known compounds *3*–*8*

All spectral data of known compounds, cabenoside G (**3**), cabenoside B (**4**), cabenoside A (**5**), cayaponoside C_{5b} (**6**), cayaponoside A_5 (**7**), and cayaponoside B_5 (**8**), were consistent with those as previously reported (Farias, Schenkel, Mayer, & Ruecker, 1993; Himeno et al., 1994; Matos et al., 1991; Nakano, Kanai, Murakami, & Takaishi, 1995a). Selected spectral data are listed below.

Cabenoside G (**3**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −271° (c 0.23, EtOH); IR (KBr) ν_{max}: 3395, 1709, 1639, 1460, 1383 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 0.85 (3H, s, H-18), 0.86 (3H, s, H-30), 0.94 (3H, d, *J* = 5.9 Hz, H-21), 1.09 (3H, s, H-28), 1.30 (3H, s, H-19), 1.51 (3H, s, H-29), 1.60 (6H, s, H-26 and -27), 3.70 (1H ,bs, H-3), 4.81 (1H, d, *J* = 7.8 Hz, H-1'), 5.01 (1H, d, *J* = 7.6 Hz, H-1'''), 5.15 (1H, d, *J* = 7.5 Hz, H-1''), 5.63 (1H, d, *J* = 5.1 Hz, H-6); ^{13}C NMR (100 MHz, pyridine-d_5): δ 16.97, 18.83, 19.18, 23.62, 24.53, 24.59, 26.18, 26.18, 26.77, 27.59, 28.22, 29.47, 30.75, 34.44, 34.77, 36.15, 36.73, 40.12, 41.00, 42.27, 43.44, 47.34, 49.69, 50.95, 62.73, 62.98, 70.36, 71.66, 71.72, 71.88, 75.24, 75.35, 75.35, 77.32, 77.79, 78.17, 78.26, 78.26, 78.44, 78.56, 82.78, 87.90, 99.65, 105.46, 107.00, 118.38, 144.32, 214.23.

Cabenoside B (**4**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −68° (c 0.20, EtOH); IR (KBr) ν_{max}: 3385, 1684, 1602, 1481, 1388 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.32 (3H, s, H-30), 1.40 (3H, s, H-18), 1.45 (6H, s, H-26 and -27), 1.46 (3H, s, H-19), 1.52 (3H, s, H-21), 1.88 (1H, d, *J* = 13.2 Hz, H-15), 2.23 (1H, m, H-15), 2.31 (3H, s, H-28), 2.66 (1H, d, *J* = 6.1 Hz, H-8), 2.85 (1H, d, *J* = 6.6 Hz, H-17), 3.11 (1H, d, *J* = 15.1 Hz, H-12), 3.22 (1H, d, *J* = 15.1 Hz, H-12), 3.93 (1H, ddd, *J* = 9.3, 7.6, 2.7 Hz, H-5'), 4.01 (1H, ddd, *J* = 9.5, 7.6, 2.7 Hz, H-5''), 4.15 (1H, dd, *J* = 9.7, 7.8 Hz, H-

2''), 4.21 (1H, m, H-3'), 4.23 (1H, m, H-2'), 4.27 (1H, m, H-4''), 4.30 (1H, t, J = 9.7 Hz, H-3''), 4.35 (1H, dd, J = 9.3, 8.5 Hz, H-4'), 4.44 (1H, m, H-6''), 4.46 (1H, m, H-6'), 4.59 (1H, m, H-6''), 4.63 (2H, m, H-22 and -6'), 5.24 (1H, d, J = 7.8 Hz, H-1''), 5.29 (1H, m, H-16), 5.35 (1H, d, J = 7.8 Hz, H-1'), 5.84 (1H, dd, J = 10.1, 6.1 Hz, H-7), 6.36 (1H, d, J = 15.6 Hz, H-24), 6.49 (1H, dd, J = 15.4, 5.3 Hz, H-23), 6.87 (1 H, d, J = 10.0 Hz, H-6), 7.20 (1H, s, H-1); ^{13}C NMR (100 MHz, pyridine-d_5): δ 11.73, 16.79, 19.26, 23.15, 26.95, 29.77, 43.71, 46.20, 47.44, 49.40, 49.68, 50.83, 55.94, 60.90, 61.46, 68.88, 69.36, 70.33, 70.76, 75.06, 75.35, 76.82, 77.22, 77.79, 78.05, 79.95, 83.58, 102.45, 106.00, 112.02, 120.94, 124.22, 125.01, 126.12, 126.68, 128.46, 140.55, 143.97, 144.45, 212.72.

Cabenoside A (**5**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −173° (c 0.23, EtOH); IR (KBr) ν_{max}: 3391, 1684, 1481 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.26 (3H, s, H-30), 1.37 (3H, s, H-18), 1.48 (9H, s, H-19, -26 and -27), 1.50 (3H, s, H-21), 2.46 (3H, s, H-28), 3.93 (1H, ddd, J = 9.0, 6.1, 2.7 Hz, H-5'), 4.16 (1H, dd, J = 9.0, 7.8 Hz, H-2'), 4.24 (1H, dd, J = 9.3, 9.0 Hz, H-3'), 4.36 (1H, dd, J = 9.3, 9.0 Hz, H-4'), 4.42 (1H, m, H-6'), 4.52 (1H, m, H-6'), 4.58 (1H, m, H-22), 5.27 (1H, m, H-16), 5.41 (1H, d, J = 7.8 Hz, H-1'), 5.84 (1H, dd, J = 10.1, 6.4 Hz, H-7), 6.34 (1H, d, J = 15.4 Hz, H-24), 6.49 (1H, dd, J = 15.4, 5.3 Hz, H-23), 6.93 (1H, d, J = 10.0 Hz, H-6), 7.19 (1H, s, H-1). ^{13}C NMR (100 MHz, pyridine-d_5): δ 11.73, 17.71, 20.37, 25.68, 26.95, 30.88, 43.71, 47.17, 48.35, 50.40, 50.63, 51.71, 56.81, 61.88, 68.88, 69.82, 70.80, 71.68, 74.76, 76.22, 78.35, 78.88, 81.64, 105.46, 114.02, 122.90, 125.33, 125.83, 127.13, 127.91, 129.18, 141.75, 145.32, 146.30, 213.47.

Cayaponoside C5b (**6**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −145° (c 0.57, EtOH); IR (KBr) ν_{max}: 3409, 1686, 1653 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.24 (3H, s, H-30), 1.29 (3H, s, H-18), 1.34 (6H, s, H-26 and -27), 1.46 (3H, s, H-19), 1.55 (3H, s, H-21), 2.15 (1H, m, H-24), 2.18 (1H, m, H-24), 2.45 (3H, s, H-28), 3.21 (1H, m, H-23), 2.42 (1H, m, H-23), 4.89 (1H, dd, J = 10.5, 7.2 Hz, H-16), 5.49 (1H, d, J = 8.0 Hz, H-1'), 5.82 (1H, dd, J = 10.2, 6.2 Hz, H-7), 6.94 (1H, d, J = 10.2 Hz, H-6), 7.24 (1H, s, H-1). ^{13}C NMR (100 MHz, pyridine-d_5): δ 11.65, 17.83, 20.20, 25.30, 26.97, 29.70, 30.07, 32.66, 38.38, 44.58, 47.11, 48.60, 49.63, 50.88, 51.50, 59.12, 61.82, 68.99, 70.59, 70.70, 74.77, 78.38, 78.84, 79.99, 105.22, 113.38, 122.93, 125.41, 126.89, 127.65, 129.16, 145.47, 146.10, 213.19, 216.01.

Cayaponoside A5 (**7**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −114° (c 0.33, EtOH); IR (KBr) ν_{max}: 3422, 1698, 1603 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.27 (3H, s, H-30), 1.29 (3H, s, H-18), 1.45 and 1.47 (6H, s, H-26 and -27), 1.46 (3H, s, H-19), 1.57 (3H, s, H-21), 1.88 (3H, s, Ac), 2.46 (3H, s, H-28), 4.92 (1H, dddd, J = 10.7, 8.7, 7.0, 4.6 Hz, H-16), 5.46 (1H, d, J = 8.0 Hz, H-1'), 5.83 (1H, dd, J = 10.0, 6.3 Hz, H-7), 6.95 (1H, d, J = 10.0 Hz, H-6), 7.24 (1H, s, H-1). ^{13}C NMR (100 MHz, pyridine-d_5): δ 11.65, 17.85, 20.22, 22.19, 25.38, 25.94, 26.00, 26.99, 32.10, 35.23, 38.38, 44.55, 47.11, 48.62, 49.66, 50.89, 51.51, 59.35, 61.79, 70.61, 70.66, 74.77, 78.40, 78.84, 80.02, 81.52, 105.19, 113.34, 122.95, 125.41, 126.88, 127.64, 129.16, 145.50, 146.08, 170.11, 213.10, 215.08.

Cayaponoside B5 (**8**) was obtained as an amorphous solid; $[\alpha]_D^{27}$ −136° (c 0.35, EtOH); IR (KBr) ν_{max}: 3436, 1684 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.32 (3H, s, H-30), 1.40 (3H, s, H-18), 1.47 (3H, s, H-19), 1.52 (3H, s, H-21), 1.80 (3H, s, CH_3-25), 2.46 (1H, m, H-28), 4.79 (1H, m, H-22), 4.95 and 4.99 (2H, s, CH_2-25), 4.31 (1H, m, H-16), 5.44 (1H, d, J = 8.0 Hz, H-1'), 5.85 (1H, dd, J = 10.0, 6.1 Hz, H-7), 6.34 (1H, dd, J = 15.9, 5.2 Hz, H-23), 6.81 (1H, d, J = 15.9 Hz, H-24), 6.94 (1H, d, J = 10.0 Hz, H-6), 7.23 (1H, s, H-1). ^{13}C NMR (100 MHz, pyridine-d_5): δ 11.67, 17.73, 18.76, 20.28, 23.60, 27.09, 44.12, 47.17, 48.52, 50.24, 50.71, 51.86, 57.10, 61.84, 70.72, 71.64, 74.81, 76.41, 78.34, 78.84, 80.40, 105.37, 113.66, 115.97, 122.90, 125.38, 127.05, 127.77, 129.23, 131.02, 133.89, 142.33, 145.47, 146.19, 213.19.

2.7. Sensory evaluation of sweetness

Sweetness relative to sucrose was evaluated by a human sensory panel as previously described (Kasai, Matsumoto, Nie, Zhou, & Tanaka, 1988). All saponins were dissolved in H_2O to give a 100 μg/ml solution. Sucrose solutions were made at graduated concentrations from 1 mg/ml to 10 μg/ml. The panelists were asked to taste a sucrose solution and estimate its total taste intensity relative to that of the sample solution. The assays were performed at least in triplicate on separate occasions.

3. Results and discussion

3.1. Isolation of sweet and bitter constituents of Wilbrandia species

The ethanolic extract from the dried root of *Wilbrandia* species was partitioned between H_2O and *n*-hexane, EtOAc, and *n*-BuOH in this order. The *n*-BuOH fraction contained a delicate sweet taste, was subjected to silica gel chromatography followed by MPLC to give three sweet compounds, **1**–**3**, along with a bitter compound, **4** (Fig. 1). In contrast, the EtOAc fraction retained a strong bitter taste. The major bitter constituents were purified by silica gel chromatography followed by preparative HPLC. Consequently, four bitter compounds, **5**–**8**, were isolated as an amorphous solid. The known compounds, **4**–**8**, were determined as cucurbitane or *nor*-cucurbitane triterpene glycosides by spectroscopic comparisons with those as previously reported (Farias et al., 1993; Nakano, Kanai, Murakami, & Takaishi, 1995b; Nihei et al., 2002; Takemoto, Arihara, Nakajima, & Okuhira, 1983a).

3.2. Structure of wilbrandiside A

Wilbrandiside A (**1**) was isolated as an amorphous solid and the molecular formula was established as $C_{48}H_{80}O_{19}$ by HRFABMS

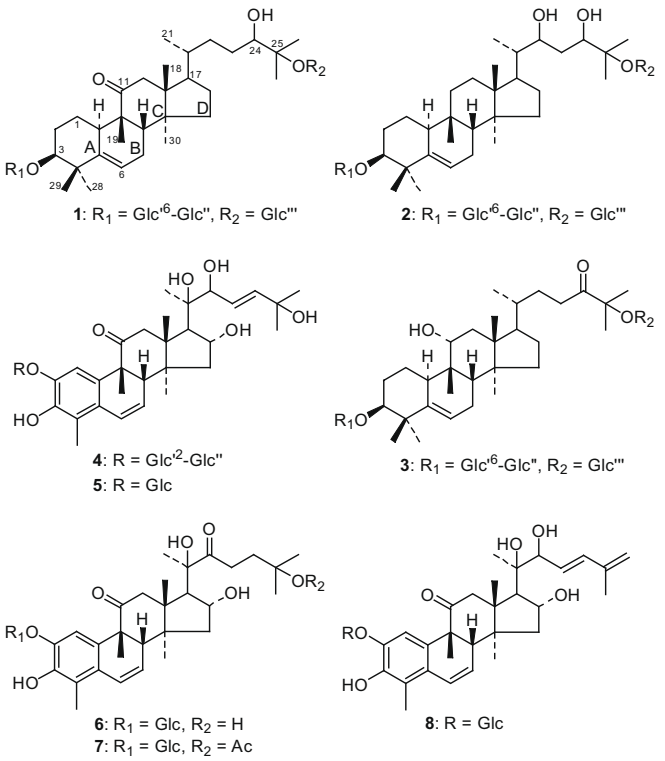

Fig. 1. Structures of wilbrandiside A (**1**), and B (**2**), and their related compounds.

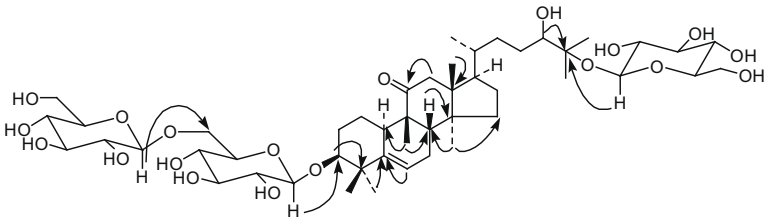

Fig. 2. Significant HMBC correlations in **1**.

experiments. The IR spectra of **1** showed strong absorptions at 3400, 1685, and 1641 cm^{-1}, which indicated the presence of hydroxyl, ketone, and olefin moieties in this structure. The observation of seven singlet methyl signals (δ_H 0.69, 0.85, 0.94, 1.03, 1.49, 1.52; δ_C 16.96, 18.17, 20.26, 22.90, 22.57, 25.77, 28.28) and three anomeric signals (δ_H 4.79, 5.13, 5.22; δ_C 97.57, 105.50, 106.97) by ^1H and ^{13}C NMR experiments led to a consideration that **1** was classified as a saponin with three sugars.

The assignment for each signal revealed in the ^1H and ^{13}C NMR spectra was performed by the two-dimensional NMR experiments including ^1H–^1H COSY, ^1H–^{13}C COSY and HMBC (Tables 1 and 2). The significant HMBC correlations are shown in Fig. 2. By ^1H–^1H COSY experiments, an oxymethine proton at 3.70 ppm (H-3) was correlated to a methine proton at 2.41 ppm (H-10) through two methylene protons at 2.41, 1.89, 1.98, and 1.61 ppm (H-1 and -2). Also, this oxymethine signal possessed four significant cross peaks with the signals of an anomeric position at C-1' (δ_H 4.79; δ_C 106.97), geminal dimethyls at C-28 and -29 (δ_H 1.03, 1.49; δ_C 25.77, 28.28), and a quaternary carbon at 41.96 ppm (C-4) in the HMBC spectra. Accordingly, these resonances were placed in the A ring of **1**. A sugar unit attached at C-3 was determined as a 1,6-linked glycoside by observing long-range couplings between C-6' and C-1'' in the HMBC spectra. In addition, each sugar was identified as a β-glucose because of all oxymethines showing large coupling constants (7.6–9.5 Hz).

In the ^1H–^1H COSY spectra, an olefinic proton at 5.49 ppm (H-6) possessed cross peaks with methylene protons at 1.74 and 2.13 ppm (H-7) that were further coupled to a methine proton at 1.77 ppm (H-8). Two key HMBC correlations between the geminal dimethyls (C-28 and -29) and a quaternary olefinic carbon at 141.30 ppm (C-5), and a singlet methyl at C-19 (δ_H 0.85; δ_C 20.26) and a methine at C-8 (δ_H 1.77; δ_C 43.92) were consistent with the structure of the B ring. An isolated methylene at 1.46 and 1.63 ppm observed in the ^1H–^1H COSY spectra and a carbonyl carbon at 213.75 ppm were assigned to C-12 and C-11, respectively, since the methyl at H-19 and the methylene at H-12 were correlated to the carbonyl carbon at C-11 in the HMBC spectra. The methyl signal (δ_H 0.69; δ_C 16.96) possessed long-range correlations with the methylene at C-12 and a methine at C-17 (δ_H 1.71; δ_C 49.99) so that this methyl signal was assigned to C-18. On the other hand, the methyl signal at C-30 (δ_H 0.94; δ_C 18.17) was connected with C-14 as two significant HMBC correlations were observed between the methyl at C-30, and the methine at C-8 and a methylene at C-15. Accordingly, the proton resonances at H-15, -16 and -17, which were shown in the ^1H–^1H COSY spectra, were allowed to make up the D ring structure of **1**. The presence of the five singlet methyls at C-18, -19, -28, -29 and -30 suggested that this compound was classified as a cucurbitane triterpenoid which was generally found in the genus of *Cucurbitaceae*.

In the ^1H–^1H COSY spectra, the methine proton at H-17 showed a cross peak with a methine at 1.40 ppm (H-20) that was further correlated to an oxymethine proton at 4.00 ppm (H-24) through two methylene protons at 1.07, 1.51, 1.67 and 1.71 ppm. These nuclei should be assigned to the side chain of cucurbitane skeleton. A deshielded quaternary carbon at C-25 (80.51 ppm) was glycosylated since the HMBC spectra revealed three significant long-range couplings with an anomeric position at C-1''' (δ_H 5.22; δ_C 97.57) (Ukita, Akihisa, Yasukawa, et al., 2002), geminal dimethyls at C-26 and -27 (δ_H 1.52; δ_C 22.57, 22.90), and the oxymethine at C-24. A glycoside attached to C-24 was confirmed by a β-glucose because every oxymethine proton possessed large coupling constants (7.8–9.0 Hz).

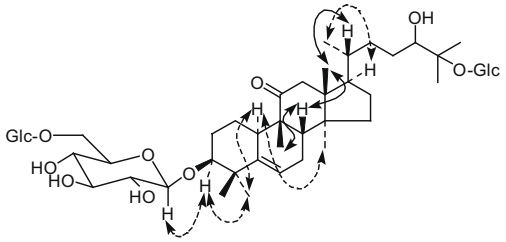

Fig. 3. Significant NOE correlations in **1**. Dashed line expresses NOE on α-face.

The stereochemistry of **1** was elucidated by NOESY experiments (Fig. 3) and vicinal coupling constants observed in the ^1H NMR spectra. The A ring conformation as a chair form was deduced from two cross peaks between H-28 and -2_α, and H-28 and -10 in the NOESY spectra. In addition, considering a small coupling constant at H-3 led to an assignment that the sugar moiety was orientated to the β-position on the A ring. The NOE observations at H-1_β, -19, -7_β, -8_β, and -18 indicated that the B/C ring junction was cis. Likewise, the C/D ring junction was expected to be trans due to two key NOEs observed between H-12_α and -30, and H-30 and -16_α (Monte, Papa, Kintzinger, & Braz-Filho, 2000).

3.3. Structure of wilbrandiside B

Wilbrandiside B (**2**) was isolated as an amorphous solid with the molecular formula, $C_{48}H_{82}O_{19}$, which was established by HRFABMS experiments. Absorptions at 3395 and 1645 cm^{-1} in IR spectra suggested being hydroxyl and olefin moieties in this structure. Both the ^1H and ^{13}C NMR spectra of **1** and **2** revealed the number of similarities so that the structure of **2** was also assumed to be a cucurbitane triterpene glycoside.

The ^1H and ^{13}C NMR assignments for **2** were carried out by ^1H–^1H COSY, ^1H–^{13}C COSY, HMBC and NOESY experiments (Tables 1 and 2). As a result, the resonances from the A to B rings, and glycosides were almost consistent with those of **1**. The large differences between **1** and **2** were derived from the signals of the C ring to the side chain. A methylene at C-11 (δ_H 1.33, 1.63; δ_C 31.36) on the C ring exhibited instead of a carbonyl, which was supported by the HMBC correlations at C-19 and the coupling at C-12 methylene protons. In addition, the inspection of the ^1H–^1H COSY spectra clarified that an oxymethine (δ_H 4.55; δ_C 68.66)

Table 3
Taste and relative sweetness for cucurbitane glycosides from the root of *Wilbrandia* species.

Compounds tested	Taste	Relative sweetness[a]
Wilbrandiside A (**1**)	Sweet	28
Wilbrandiside B (**2**)	Sweet	6
Cabenoside G (**3**)	Sweet	5
Cabenoside B (**4**)	Bitter	–[b]
Cabenoside A (**5**)	Bitter	–
Cayaponoside C_{5b} (**6**)	Bitter	–
Cayaponoside A_5 (**7**)	Extremely Bitter	–
Cayaponoside B_5 (**8**)	Bitter	–

[a] Sucrose = 1.
[b] not tested.

was located at C-22. Consequently, the structure of **2** was determined as an analogue of **1** as shown in Fig. 1.

3.4. Biological properties of cucurbitane triterpenoids from Wilbrandia species

It has been reported that several cucurbitane glycosides such as mogrosides possess strong sweet taste as compared to sucrose (Takemoto, Arihara, Nakajima, & Okuhira, 1983b; Takemoto et al., 1983a). Therefore, the human sensory panel experiments were performed using cucurbitane glycosides from *Wilbrandia* species (Table 3), in order to evaluate their sweetness. As a result, cucurbitane glycosides, **1**–**3**, were sweet although *nor*-cucurbitane glycosides, **4**–**8**, have bitter taste. Interestingly, compound **1** was 28 times sweeter than sucrose without any bitterness. Structure-taste relationship amongst cucurbitane glycosides was well-discussed in a previous report (Kasai et al., 1987, 1988). It suggested that the compound having a carbonyl on the C ring tended to decrease its sweetness, whereas this functional group was found in the structure of **1**. However, it should be mentioned that amongst sweet compounds, **1**–**3**, two glycosylations took place in the A ring and the side chain. This bidentate glycoside was possibly essential to the sweet taste and a sweet-taste relationship has been proposed (Kasai et al., 1987, 1988). On the other hand, the aromatisation of the A ring as shown in *nor*-cucurbitane glycosides would enhance their bitterness.

Besides sweet and bitter properties of cucurbitane and *nor*-cucurbitane glycosides, they contain attractive biological significances as antiinflammatories (Almeida, Rao, & Matos, 1992) and anticarcinogens (Takasaki et al., 2003; Ukita, Akihisa, Tokuda, et al., 2002). Mogroside V, an analogue of **1** and **2**, has not been shown to be mutagenic or to possess acute toxicity, suggesting that similar cucurbitanes may be harmless (Makapugay, Nanayakkara, Soejarto, & Kinghorn, 1985). Taking into account not only the need of low-caloric sweeteners for more than 150 million people in the United States (Nabors, 2001), but also their extensive biological activities it is suggested that cucurbitane triterpenoids might have potential as multifunctional food additives from natural origins. In addition, it should be noted that the compounds we characterised as antifeedants against *P. gossypiella* and *H. virescens* larvae taste bitter, but all bitter tasting compounds isolated are not antifeedants.

Acknowledgements

We are indebted to Dr. J.M. Pines, Goeldi Museum, for identification of the plant material and Mr. J.M. Takeda for his assistance to obtain the plant material.

References

Almeida, F. R. C., Rao, V. S. N., & Matos, M. E. O. (1992). Anti-inflammatory, antitumor and antifertility effects in rodents of two *nor*-cucurbitacin glucosides from *Wilbrandia* species. *Phytotheraphy Research, 6*, 189–193.
Braga, R. (1960). *Plantas do nordeste, especialmente do ceará*. Fortaleza: Imperesa Oficia. pp. 98–99.
Butterworth, J. H., & Morgan, E. D. (1968). Isolation of a substance that suppresses feeding in locusts. *Chemical Communications*, 23–24.
Farias, M. R., Schenkel, E. P., Mayer, R., & Ruecker, G. (1993). Cucurbitacins as constituents of *Wilbrandia ebracteata*. *Planta Medica, 59*, 272–275.
Himeno, E., Nagao, T., Honda, J., Okabe, H., Irino, N., & Nakasumi, T. (1994). Studies on the constituents of the root of *Cayaponia tayuya* (Vell.) Cogn. III. Structures of cayaponosides, 29-*nor*-1,2,3,4,5,10-hexadehydrocucurbit-6-ene glucosides. *Chemical & Pharmaceutical Bulletin, 42*, 2370–2372.
Kasai, R., Matsumoto, K., Nie, R. L., Morita, T., Awazu, A., Zhou, J., et al. (1987). Sweet and bitter cucurbitane glycosides from *Hemsyleya carnosiflora*. *Phytochemistry, 26*, 1371–1376.
Kasai, R., Matsumoto, K., Nie, R. L., Zhou, J., & Tanaka, O. (1988). Glycosides from Chinese medicinal plant, *Hemsyleya panacis-scandens*, and structure-teste relationship of cucurbitane glycosides. *Chemical & Pharmaceutical Bulletin, 36*, 234–243.
Kubo, I. (1993). Insect control agents from tropical plants. In K. R. Downum, J. T. Romeo, & H. A. Stafford (Eds.). *Phytochemical potential of tropical plants: Recent advances in phytochemistry* (Vol. 27, pp. 133–151). New York: Plenum Press.
Kubo, I., Lee, Y. W., Balogh-Nair, V., Nakanishi, K., & Chapya, A. (1976). Structure of ajugarins. *Journal of Chemical Society, Chemical Communications*, 949–950.
Makapugay, H. C., Nanayakkara, N. P. D., Soejarto, D. D., & Kinghorn, A. D. (1985). High-performance liquid chromatographic analysis of the major sweet principle of Lo Han Kuo fruits. *Journal of Agricultural and Food Chemistry, 33*, 348–350.
Matos, M. E. O., Machado, M. I. L., Craveiro, A. A., Matos, F. J. A., & Braz-Filho, R. (1991). *Nor*-Cucurbirtacin glucosides from *Wilbrandia* species. *Phytochemistry, 30*, 1020–1023.
Monte, F. J. Q., Papa, S. M. A., Kintzinger, J. P., & Braz-Filho, R. (2000). Total assignment of ^{1}H and ^{13}C NMR spectra of two isomeric cucurbitane triterpenoids. *Magnetic Resonance in Chemistry, 38*, 312–809.
Nabors, L. O. (2001). Alternative sweeteners: An overview. In L. O. Nabors (Ed.), *Alternative sweeteners* (pp. 1–12). New York: Marcel Dekker.
Nakano, K., Kanai, Y., Murakami, K., & Takaishi, Y. (1995a). Cucurbitacin glycosides from cabeça-de-negro. *Phytochemistry, 39*, 209–211.
Nakano, K., Kanai, Y., Murakami, K., & Takaishi, Y. (1995b). Cucurbitacin glycosides from *Caput nigri*. *Phytochemistry, 39*, 205–207.
Nihei, K., Hanke, F. J., Asaka, Y., Matsumoto, T., & Kubo, I. (2002). Insect antifeedants from tropical plants II: Structure of zumsin. *Journal of Agricultural and Food Chemistry, 50*, 5048–5052.
Takasaki, M., Konoshima, T., Murata, Y., Sugiura, M., Nishino, H., Tokuda, H., et al. (2003). Anticarcinogenic activity of natural sweeteners, cucurbitane glycosides, from *Momordica grosvenori*. *Cancer Letters, 198*, 37–42.
Takemoto, T., Arihara, S., Nakajima, T., & Okuhira, M. (1983a). Studies on the constituents of Fructus momordicae. I. On the sweet principle. *Yakugaku Zasshi, 103*, 1151–1154.
Takemoto, T., Arihara, S., Nakajima, T., & Okuhira, M. (1983b). Studies on the constituents of *Fructus momordicae* III. Structure of mogrosides. *Yakugaku Zasshi, 103*, 1167–1173.
Ukita, M., Akihisa, T., Tokuda, H., Toriumi, M., Mukainaka, T., Banno, N., et al. (2002). Inhibitory effects of cucurbitane glycosides and other triterpenoids from the fruit of *M. grosvenori* on Epstein-Barr virus early antigen induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate. *Journal of Agricultural and Food Chemistry, 50*, 6710–6715.
Ukita, M., Akihisa, T., Yasukawa, K., Tokuda, H., Toriumi, M., Koike, K., et al. (2002). Anti-inflammatory and anti-tumor-promoting effects of cucurbitane glycoside from the root of *Bryonia dioica*. *Journal of Natural Products, 65*, 179–183.
Zhang, M., & Kubo, I. (1992). Possible function of ecdysteroid-22-O-acyltransferase in the larval gut of tobacco budworm, *H. virescens*. *Journal of Chemical Ecology, 18*, 1139–1149.