Two New Cucurbitacin Glucosides, Opercurins A and B, from the Brazilian Folk Medicine "Buchinha" (*Luffa operculata*)

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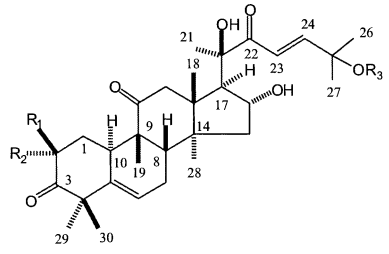
The structures of two new cucurbitacin glucosides designated opercurins A (1) and B (2), isolated from the fruit of *Luffa operculata*, have been confirmed by extensive spectroscopic investigation.

Key words opercurin; *Luffa operculata*; Buchinha; Cucurbitaceae; cucurbitacin

In a previous report,[1] two novel cucurbitacins with a new skeleton designated as neocucurbitacins A and B were reported to be isolated from the dichloromethane extract of the fruit of *Luffa operculata* (L.) COGN. (Cucurbitaceae), a biennial medicinal plant that grows in the Amazon region. The plant is commonly known as "Buchinha" or "Bucha-dos-paulistas" in Brazil, and the fruit's aqueous extract has long been used as a remedy for urethritis and empyema, and to treat edema.[2] Further investigation of EtOAc extract facilitated the isolation of two new cucurbitacin glucosides, designated opercurins A (**1**) and B (**2**), as well as two known cucurbitacin glucosides, arvenins I (**3**)[3,4] and III (**4**).[4] The structural elucidation of compounds **1** and **2** is reported herein.

Opercurin A (**1**) formed a colorless amorphous powder and was determined by high resolution (HR)-FAB-MS to have the molecular formula $C_{38}H_{55}O_{13}$ with a quasimolecular ion at *m/z* 719.3645 (Calcd 719.3643) $(M-H)^-$. The IR (3439, 1725, 1690 cm^{-1}) spectrum suggested the presence of hydroxyl and carbonyl groups. The ^{1}H-NMR spectrum of **1** (Table 1) exhibited 50 nonexchangeable protons, including nine tertiary methyl groups (δ 0.86, 1.13, 1.26, 1.29, 1.36, 1.38, 1.53, 1.56, 2.00) and three olefinic protons (δ 5.82 [br s], 6.82, 6.96 [each d, J=15.6 Hz]). The ^{13}C-NMR spectrum of **1** (Table 2) showed nine methyls, five methylenes, including an oxygenated carbon, ten methines, including seven oxygenated carbons, six quaternary carbons, including two oxygenated carbons, four olefinic carbons (δ 122.4, 122.7, 139.1, 151.5) and four carbonyl carbons (δ 171.9, 205.3, 214.6, 216.7). The presence of a methyl group (δ 2.00) and ester carbonyl group (δ 171.9), as well as an oxygenated methylene carbon (δ 62.6) and five oxygenated methine carbons (δ 71.4, 74.6, 77.9, 78.25, 100.9) suggested acetoxyl and hexosyl moieties in the molecule, respectively. Enzymatic hydrolysis of **1** revealed that the hexosyl moiety was a β-D-glucopyranoside. These data were closely similar to those of the known cucurbitacin glucoside, arvenin I (**3**). The heteronuclear multiple-bond correlation (HMBC, $^{2\text{-}3}J_{C-H}$= 6.3 Hz) spectrum of **1** revealed the presence of the same planar structure as **3** (Fig. 1). Thus, the structure of **1** was determined to be a stereoisomer of **3**.



1 : R_1 = H, R_2 = O-β-D-glucose, R_3 = Ac

3 : R_1 = O-β-D-glucose, R_2 = H, R_3 = Ac

4 : R_1 = O-β-D-glucose, R_2 = H, R_3 = H

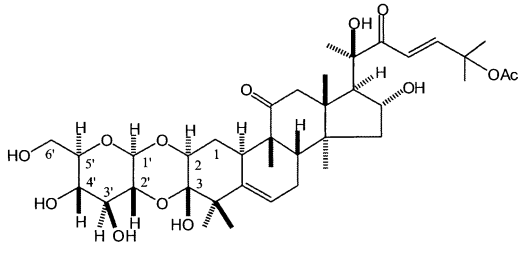


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Chart 1

The stereochemistry of **1** was confirmed by nuclear Overhouser exchange spectroscopy (NOESY) spectrum, as shown in Fig. 2. The H-2 showed cross peaks to H_3-19 and H_3-30, whereas H-10 showed cross peaks to H_3-29. These data indicate that H-2 takes the β-orientation. Further, key NOE correlations of **1** revealed the same stereochemistry at the B–D ring and side chain as those of known cucurbitacins. Therefore, the structure of opercurin A, a diastereoisomer of **3** at the C-2 position, was determined as shown in **1**.

Opercurin B (**2**) formed a colorless amorphous powder and was determined by HR-FAB-MS to have the molecular formula $C_{38}H_{55}O_{13}$ with a quasimolecular ion at *m/z* 719.3643 (Calcd 719.3643) $(M-H)^-$. The IR (3451, 1725, 1690 cm^{-1}) spectrum suggested the presence of hydroxyl and carbonyl groups. The ^{1}H- and ^{13}C-NMR spectra of **2** showed almost the same signal patterns as those of **3**, with the exception of the appearance of a signal for a hemiketal group (δ 97.1) rather than the disappearance of a signal for a carbonyl group (C-3). The partial structure of the A-ring was deduced from the HMBC spectrum (Fig. 1). The oxygenated methine

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Table 1. ^{1}H-NMR Chemical Shifts of Opercurins A, B, Arvenins I and III (**1**—**4**)[a] in CD_3OD

Position		**1**[b]	**2**[b]	**3**[b]	**4**[c]
1	(α)	1.70 ddd (6.0, 11.5, 14.7)	1.48 dd (4.1, 11.9)	2.21 ddd (3.7, 5.5, 13.3)	2.22 ddd (3.7, 5.6, 13.3)
	(β)	2.08 dt (4.6, 14.7)	1.60 q (11.9)	1.30 q (13.3)	1.31 q (13.3)
2		4.42 dd (4.6, 11.5)	4.02 dd (4.1, 11.9)	4.89 dd (5.5, 13.3)	4.91 dd (5.6, 13.3)
6		5.82 br s	5.70 br s	5.81 br s	5.81 br s
7	(α)	2.05 m	1.95 m	2.00 m	2.02 m
	(β)	2.44 m	2.39 m	2.40 m	2.41 m
8		1.99 d (11.7)	1.92 d (8.2)	1.95 d (9.2)	1.97 d (7.8)
10		3.15 m	2.68 br d (11.9)	3.02 br dd (3.7, 13.3)	3.03 br dd (3.7, 13.3)
12		3.32 d (14.7)	3.29 d (14.3)	3.39 d (14.7)	3.42 d (15.1)
		2.51 d (14.7)	2.48 d (14.3)	2.57 d (14.7)	2.62 d (15.1)
15	(α)	1.42 br d (12.8)	1.39 br d (12.8)	1.41 br d (12.8)	1.40 br d (12.8)
	(β)	1.84 br dd (9.2, 12.8)	1.82 br dd (9.2, 12.8)	1.85 br dd (9.2, 12.8)	1.85 br dd (9.1, 12.8)
16		4.55 dd (8.3, 9.2)	4.53 dd (7.3, 9.2)	4.54 dd (7.8, 9.2)	4.48 dd (7.3, 9.1)
17		2.53 d (8.3)	2.55 d (7.3)	2.55 d (7.3)	2.61 d (7.3)
18	(3H)	0.86 s	0.84 s	0.87 s	0.91 s
19	(3H)	1.13 s	1.08 s	1.03 s	1.03 s
21	(3H)	1.38 s	1.38 s	1.40 s	1.39 s
23		6.82 d (15.6)	6.82 d (15.6)	6.82 d (15.6)	6.83 d (15.6)
24		6.96 d (15.6)	6.96 d (15.6)	6.97 d (15.6)	6.97 d (15.6)
26	(3H)	1.56 s	1.56 s	1.56 s	1.31 s
27	(3H)	1.53 s	1.53 s	1.54 s	1.31 s
28	(3H)	1.36 s	1.31 s	1.36 s	1.38 s
29	(3H)	1.29 s	1.13 s	1.32 s	1.32 s
30	(3H)	1.26 s	1.23 s	1.27 s	1.27 s
1′		4.35 d (7.8)	4.36 d (7.9)	4.39 d (7.8)	4.32 d (7.8)
2′		3.22 dd (7.8, 9.2)	3.48 m	3.23 dd (7.8, 9.2)	3.2—3.4 m
3′		3.36 t (9.2)	3.48 m	3.35 t (9.2)	3.2—3.4 m
4′		3.28 t (9.2)	3.37 m	3.24 m	3.2—3.4 m
5′		3.26 ddd (2.3, 6.4, 8.9)	3.35 m	3.24 m	3.2—3.4 m
6′		3.63 dd (6.4, 11.9)	3.68 dd (4.9, 11.9)	3.65 dd (5.9, 11.9)	3.65 dd (6.0, 11.5)
		3.81 dd (2.3, 11.9)	3.85 dd (1.8, 11.9)	3.88 dd (1.8, 11.9)	3.84 dd (1.8, 11.5)
Ac	(3H)	2.00 s	2.00 s	2.01 s	

a) *J* values (in Hz) in parentheses. *b*) 600 MHz. *c*) 500 MHz.

protons at δ 4.02 (H-2) and 3.48 (H-2′) correlated to the hemiketal group at δ 97.1, which was further correlated to two methyl groups at δ 1.13 (H_3-29) and 1.23 (H_3-30). This indicated that **2** had an ether linkage with C-3 of the A-ring and C-2 of the glucose moiety. In order to confirm the structure of **2**, a chemical transformation under acidic conditions was carried out. The product was analyzed by TLC and HPLC and identified as **3**.

The stereochemistry of C-3 was elucidated from the NOESY spectrum in acetone-d_6, as shown in Fig. 2. The hydroxyl group at δ 4.50 (C-3) showed cross peaks to H-1β, H_3-30 and H-2, whereas the H-2 showed cross peaks to H_3-29 and H-1. These data indicate a trans conformation of H-2 and the hydroxyl group attached to C-3. Thus, the structure of opercurin B was established as shown in **2**.

A large number of cucurbitacin derivatives have been isolated from the Cucurbitaceae family. However, **2** is the first example of a hemiketal-type cucurbitacin.

Experimental

General Procedures Optical rotation was measured using a JASCO DIP-370 (Tokyo, Japan) spectrometer. FAB-MS and HR-FAB-MS spectra were obtained on a JEOL JMS-SX102 (Tokyo, Japan) spectrometer. UV and IR spectra were recorded on a Hitachi U-2000 (Tokyo, Japan) spectrophotometer and a JASCO IR-5300 spectrophotometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL A-500 and A-600 spectrometers at 500 and 600 MHz, and at 125 and 150 MHz, respectively. Tetramethylsilane was used as an internal standard for the ^{1}H- and ^{13}C-NMR measurements. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). HPLC was performed on a column of LiChrospher Si 60 (250×10 mm i.d., Merck) and Inertsil ODS-3 (250×4.6 mm i.d., GL Sciences Inc., Tokyo, Japan). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected under UV light.

Plant Materials The fruits of *Luffa operculata* were collected in Belen, Brazil in 1996. A voucher specimen has been deposited at the National Institute of Health Sciences, Japan.

Extraction and Isolation The fruit of *Luffa operculata* (200 g) was crushed and extracted using MeOH (1.5 l×3). The extract (16.4 g) was dissolved in CH_2Cl_2, and the CH_2Cl_2 insoluble fraction (7.8 g) was dissolved in MeOH. The MeOH soluble fraction (3.9 g) was separated by column chromatography using Sephadex LH-20 eluted with $CHCl_3$–MeOH (2 : 1). It was then subjected to silica gel column chromatography and eluted in three fractions: $CHCl_3$–MeOH (5 : 1), $CHCl_3$–MeOH (4 : 1) and MeOH. The $CHCl_3$–MeOH (5 : 1) fraction (790 mg) was further subjected to low-pressure liquid chromatography (LPLC) eluted with 70% MeOH to afford arvenin I (**3**, 700 mg). The obtained residue (49 mg) was subjected to HPLC eluted with a $CHCl_3$–MeOH (10 : 1) solvent system to afford opercurin A (**1**, 7 mg) and opercurin B (**2**, 9 mg). The $CHCl_3$–MeOH (4 : 1) fraction (390 mg) was subjected to LPLC eluted with 70% MeOH to afford arvenin III (**4**, 10 mg).

Opercurin A (**1**): Colorless amorphous powder. $[\alpha]_D^{20}$ −3.73° (c=0.51, acetone). IR ν_{max}^{KBr} cm^{-1}: 3439 (OH), 1725 (CO) and 1690 (CO). UV λ (log ε): 224 sh (4.40), 250 sh (3.87). Negative FAB-MS *m*/*z*: 719 [M−H]$^-$, HR-FAB-MS *m*/*z*: Calcd for $C_{38}H_{55}O_{13}$: 719.3643, Found: 719.3645. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Opercurin B (**2**): Colorless amorphous powder. $[\alpha]_D^{20}$ +45.4° (c=1.42, acetone). IR ν_{max}^{KBr} cm^{-1}: 3451 (OH), 1725 (CO) and 1690 (CO). UV λ (log ε): 224 sh (4.36), 248 sh (3.76). Negative FAB-MS *m*/*z*: 719 [M−H]$^-$, HR-FAB-MS *m*/*z*: Calcd for $C_{38}H_{55}O_{13}$: 719.3643, Found: 719.3643. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Enzymatic Hydrolysis of 1 A solution of opercurin A (**1**) (1.6 mg) in H_2O (1.0 ml) and β-D-glucosidase (10 mg) from almond was incubated at 37 °C for 25 h. The solution was subsequently washed with EtOAc and the

Table 2. ^{13}C-NMR Chemical Shifts of Opercurins A, B, Arvenins I and III (**1**—**4**)[a] in CD_3OD

Position	**1**[b]	**2**[b]	**3**[b]	**4**[c]
1	25.8	29.5	35.9	35.9
2	78.33	77.0	79.6	79.6
3	214.6	97.1	213.3	213.3
4	50.9	46.6	52.4	52.4
5	139.1	141.5	141.7	141.6
6	122.4	120.6	121.4	121.3
7	25.0	24.7	24.9	24.8
8	43.8	44.5	44.1	44.1
9	51.0	50.2	49.9	49.9
10	32.3	34.9	35.0	35.0
11	216.7	216.3	215.7	215.6
12	49.7	49.6	49.7	49.8
13	51.8	51.8	51.7	51.8
14	49.0	49.1	49.1	49.0
15	46.7	46.6	46.5	46.6
16	71.9	71.9	71.8	71.6
17	60.2	60.0	60.2	60.2
18	20.8	20.7	20.8	20.7
19	18.77	20.3	20.1	20.1
20	80.3	80.3	80.2	79.9
21	25.4	25.3	25.6	25.4
22	205.3	205.2	205.4	205.0
23	122.7	122.7	122.6	121.2
24	151.5	151.5	151.5	155.3
25	81.1	81.1	81.1	71.4
26	26.5	26.4	26.5	29.2
27	26.8	26.9	26.8	29.2
28	18.76	19.4	18.9	19.4
29	28.6	25.3	29.4	29.3
30	23.8	19.9	21.8	21.8
1′	100.9	100.3	104.3	104.3
2′	74.6	73.7	75.4	75.4
3′	77.9	75.0	77.9	77.9
4′	71.4	71.9	71.5	71.4
5′	78.25	80.0	78.2	78.2
6′	62.6	62.5	62.9	62.9
Ac	21.9	21.9	21.9	
	171.9	171.9	171.9	

a) Assignments were made from the HMQC and HMBC spectra. *b*) 150 MHz. *c*) 125 MHz.

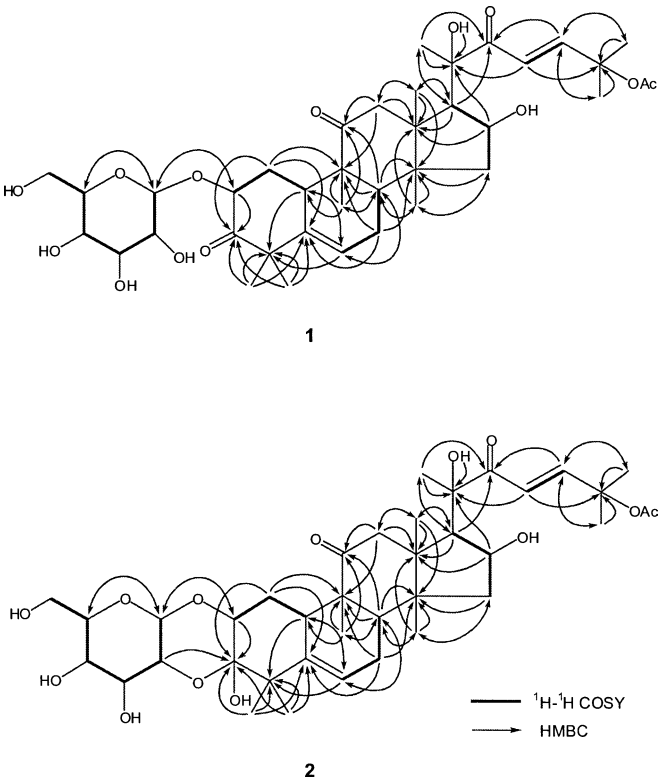

Fig. 1. ^{1}H–^{1}H and Long-Range ^{13}C–^{1}H Correlations of **1** and **2**

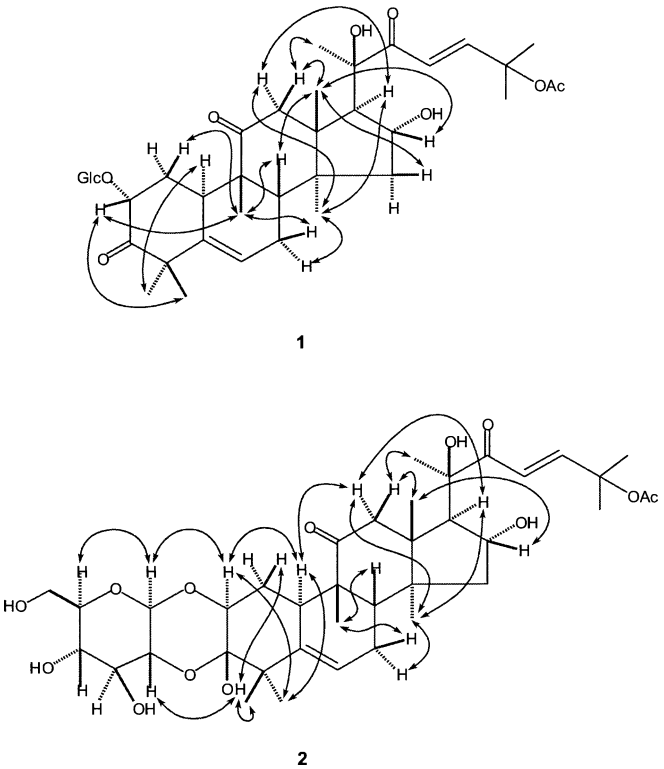

Fig. 2. NOEs of **1** and **2**

aqueous layer was evaporated. The obtained residue revealed a glucose spot upon silica gel TLC analysis (*Rf* 0.18, $CHCl_3$–MeOH–H_2O, 5 : 2 : 0.1). The residue was converted into a thiazolidine derivative and again analyzed by silica gel TLC (*Rf* 0.49 and 0.38, $CHCl_3$–MeOH–H_2O, 15 : 6 : 1).[5] Authentic thiazolidine derivatives obtained from D- and L-glucose showed spots at *Rf* 0.49 and 0.38, and 0.45, respectively.

Treatment of 2 with 10% H_2SO_4: Formation of arvenin I (3) A solution of opercurin B (**2**) (1.0 mg) in 10% H_2SO_4 (0.5 ml) and MeOH (0.5 ml) was maintained at room temperature for 20 min. The reaction mixture was neutralized using 10% NaOH and extracted with EtOAc. The EtOAc layer was subjected to TLC ($CHCl_3$–MeOH, 5 : 1) and HPLC analysis, revealing the presence of arvenin I (**3**, t_R, 8.5 min). HPLC conditions: column, Inertsil ODS-3 (250×4.6 mm, GL Sciences Inc.); mobile phase, 0.1% trifluoroacetic acid in CH_3CN : H_2O (2 : 3, v/v); flow rate, 1.0 ml/min; UV detection, 254 nm.

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