Cucurbitane-Type Triterpenoids from the African Plant *Momordica balsamina*

Cátia Ramalhete,[†] Tayyab A. Mansoor,[†] Silva Mulhovo,[‡] Joseph Molnár,[§] and Maria-José U. Ferreira*[,†]

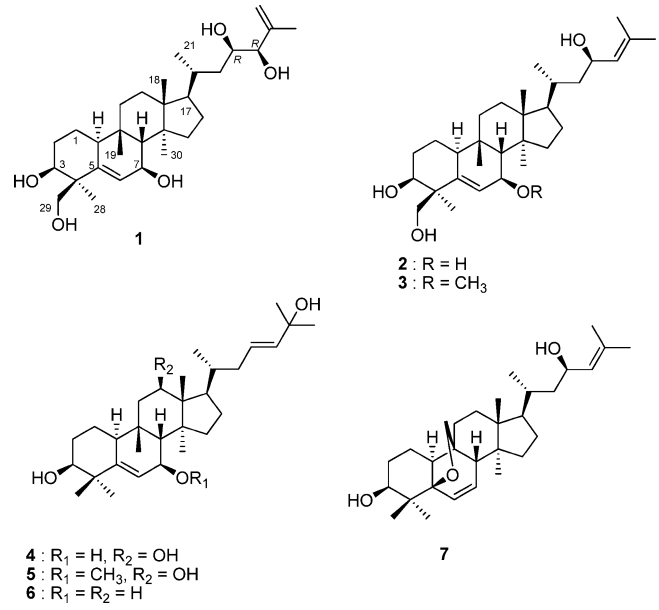
iMed-UL, Faculdade de Farmácia, Universidade de Lisboa, Avenida das Forças Armadas, 1600-083 Lisboa, Portugal, Instituto Superior Politécnico de Gaza (ISPG), Chokwe, Mozambique, and Department of Medical Microbiology and Immunobiology, University of Szeged, H-6720, Szeged, Hungary

Received July 27, 2009

Phytochemical investigation of the aerial parts of *Momordica balsamina* led to the isolation of five new cucurbitane-type triterpenoids (**1**−**5**) and two known analogues (**6**, **7**). Their structures were elucidated on the basis of spectroscopic methods including 2D NMR experiments (COSY, HMQC, HMBC, and NOESY). The new compounds feature unusual oxidation patterns in the cucurbitane skeleton, such as at C-29 (**1**−**3**) and C-12 (**4**, **5**). Compounds **1**−**4**, **6**, and **7** were evaluated for in vitro cytotoxicity against human breast cancer cells (MCF-7), using the MTT assay.

Momordica balsamina L. (Cucurbitaceae), commonly known as African pumpkin, is a vegetable widespread in tropical and subtropical regions. Due to its nutritional quality, *M. balsamina* is used largely as food, mainly in sub-Saharan Africa.[1] It has also been widely used in traditional medicine in Africa to treat various disease symptoms, mostly diabetes and malaria.[2−4] Previous investigations revealed that crude extracts of the leaves and fruits had hypoglycemic effects, as well as antimalarial activity.[5−8] The isolation of some phenylpropanoids and diterpenes was also reported.[9,10] Many cucurbitacins have been isolated from *Momordica* species, mainly from *M. charantia*. Cucurbitacins are highly oxygenated triterpenes of interest due mainly to their cytotoxic, hepatoprotective, anti-inflammatory, cardiovascular, and antidiabetic activities.[11]

Continuing our search for plant-derived bioactive compounds,[12−18] herein we report the isolation and structural elucidation of seven cucurbitane-type triterpenoids, including five new compounds (**1**−**5**) and the known compounds cucurbita-5,23(*E*)-diene-3β,7β,25-triol (**6**)[19] and karavilagenin E (**7**),[20] from a MeOH extract of *M. balsamina*. The in vitro effects of compounds **1**−**4**, **6**, and **7** on the growth of the human breast cancer cell line MCF-7 are also reported.



Results and Discussion

The air-dried, powdered aerial parts of *M. balsamina* were exhaustively extracted with methanol. Repeated column chromatographic fractionation and further purification by HPLC of the EtOAc-soluble part of the methanol extract yielded compounds **1**−**7**.

Compound **1**, named balsaminapentaol, was obtained as an amorphous powder with $[\alpha]_D^{26}$ +85 (*c* 0.11, MeOH). Its IR spectrum showed the presence of OH groups (3408 cm^{-1}) and an exocyclic double bond (1651 cm^{-1}). The low-resolution ESIMS of compound **1** exhibited a pseudomolecular ion at *m*/*z* 513 [M + Na]$^+$. The molecular formula was deduced as $C_{30}H_{50}O_5$, from its ESI-TOF-HRMS spectrum, which showed a pseudomolecular [M + Na]$^+$ ion at *m*/*z* 513.3553 (calcd for $C_{30}H_{50}O_5$, 513.3550), indicating the presence of six degrees of unsaturation. The ^{1}H NMR spectrum of **1** displayed signals due to six methyl groups: three singlets corresponding to tertiary methyl groups (δ_H 0.76, 1.05, 0.96), a doublet of a secondary methyl at δ_H 0.92 (3H, d, J = 6.5 Hz), and one vinylic methyl group (δ_H 1.70). The ^{1}H NMR spectrum also showed signals assignable to one diastereotopic methylene group [δ_H 3.65 (1H, d, J = 11.0 Hz) and 3.95 (1H, d, J = 11.0 Hz)] and four oxymethine protons [δ_H 3.63 (1H, ddd, J = 1.86, 7.0, 10.8 Hz); 3.74 (1H, d, J = 7.0 Hz); 3.82 (1H, br s); 3.94 (1H, br s)]. Furthermore, vinylic NMR signals of a trisubstituted double bond at δ_H 5.74 (1H, d, J = 4.6 Hz) and a terminal double bond at δ_H 4.78 (1H, br s) and 4.84 (1H, br s) were also observed. The ^{13}C NMR and DEPT spectra displayed 30 carbon signals corresponding to six methyl groups, nine methylenes (including an oxygenated one at δ_C 69.5 and one sp^2 carbon at δ_C 114.1), nine methines (four oxygenated at δ_C 68.5, 70.9, 75.3, and 81.6 and one sp^2 at δ_C 123.2), and six quaternary carbons (two olefinic carbons at δ_C 145.2 and 146.7). The data indicated a tetracyclic triterpenic scaffold for **1** with an OH group on one of the geminal methyl groups on ring A. This was evidenced by the relative downfield signals of H-3 (δ_H 3.82) and C-4 (δ_C 45.3) and upfield of Me-28 (δ_H 0.96), which usually resonate at $\delta_H \cong 3.50$, $\delta_C \cong 42.3$, and $\delta_H \cong 1.00$, respectively. Further structural details were obtained by two-dimensional NMR experiments (COSY, HMQC, and HMBC), which, coupled with literature data, allowed unambiguous assignment of all carbon signals (Table 2). The ^{1}H−^{1}H COSY (^2J, ^3J, and ^4J couplings) and HMQC experiments revealed the following key fragments: −CH$_2$−CH(OH)− (A); −CH−C(C)═CH−CH(OH)−CH− (B); −CH$_2$−CH(OH)−CH(OH)− (C); −C(CH$_3$)═CH$_2$ (D) (Figure 1). The heteronuclear $^2J_{C-H}$ and $^3J_{C-H}$ correlations displayed in the HMBC spectrum of **1** indicated the location of the OH groups

* To whom correspondence should be addressed. Tel: 351-21-7946475. Fax: 351-21-7946470. E-mail: mjuferreira@ff.ul.pt.
[†] Universidade de Lisboa.
[‡] Instituto Superior Politécnico de Gaza.
[§] University of Szeged.

10.1021/np900457u CCC: $40.75
Published on Web 10/01/2009

Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**5** (J in Hz; 400 MHz, MeOD)

position	**1**[a]	**2**	**3**	**4**	**5**
1	1.57 m; 1.70 m	1.56 m; 1.70 m	1.54 m; 1.70 m	1.61 m; 1.65 m	1.63 m; 1.70 m
2	1.73 m; 1.85 m	1.73 m; 1.89 m	1.73 m; 1.85 m	1.70 m; 1.90 m	1.71 m; 1.98 m
3	3.82 br s	3.83 br s	3.82 br s	3.49 br s	3.49 br s[b]
6	5.74 d (4.6)	5.74 d (4.4)	5.80 d (4.8)	5.7 4 d (4.5)	5.78 d (4.9)
7	3.94 br s	3.95 br s	3.49 d (3.7)	3.94 d (4.9)	3.49 br s[b]
8	1.98 s	1.98 s	2.06 s	1.90 s	1.98 s
10	2.33 d (1.9, 12.0)	2.33 d (10.4)	2.33 d (10.4)	2.32 m[b]	2.33 m[b]
11	1.49 m; 1.69 m	1.47 m; 1.70 m	1.49 m; 1.70 m	1.34 m; 1.89 m	1.34 m; 1.88 m
12	1.54 m; 1.74 m	1.55 m; 1.73 m	1.54 m; 1.75 m	3.86 dd (5.0, 11.4)	3.86 dd (4.9, 11.4)
15	1.32 m; 1.40 m	1.32 m; 1.40 m	1.34 m; 1.38 m	1.33 m; 1.56 m	1.36 m; 1.53 m
16	1.37 m; 1.89 m	1.39 m; 1.90 m	1.38 m; 1.90 m	1.64 m; 1,90 m	1.63 m; 1,92 m
17	1.40 m	1.46 m	1.48 m	1.88 m	1.88 m
18	0.96 s	0.97 s	0.97 s	0.89 s	0.92 s
19	1.05 s	1.05 s	0.98 s	1.08 s	1.01 s
20	1.71 m	1.48 m	1.48 m	1.81 m	1.83 m
21	0.92 d (6.5)	0.96 d (6.3)	0.97[b]	1.02 d (6.8)	1.02 d (6.8)
22	0.89 m; 1.49 m	0.95 m; 1.63 m	0.95 m; 1.63 m	1.75 m; 2.29 m	1.75 m; 2.31 m
23	3.63 ddd (1.86; 7.0; 10.8)	4.41 td (3.1; 9.5)	4.41 td (2.8; 9.6)	5.58 m	5.58 m
24	3.74 d (7.0)	5.16 d (8.5)	5.16 d (8.8)	5.58 m	5.58 m
26	4.78 br s 4.84 br s	1.66 s	1.66 s	1.25 s	1.26 s
27	1.70 s	1.70 s	1.69 s	1.25 s	1.26 s
28	0.96 s	0.97 s	0.96 s	1.01 s	1.01 s
29a	3.65 d (11.0)	3.65 d (11.0)	3.65 d (10.8)	1.18 s	1.18 s
29b	3.95 d (11.0)	3.95 d (11.0)	3.95 d (10.9)		
30	0.76 s	0.76 s	0.77 s	0.74 s	0.75 s
7-OCH$_3$			3.33 s		3.33 s

[a] Recorded at 500 MHz, in MeOD. [b] Overlapped signals.

Table 2. ^{13}C NMR Data (δ) of Compounds **1**−**5** (100 MHz, MeOD)

position	**1**	**2**	**3**	**4**	**5**
1	22.0 t	22.0 t	22.0 t	22.5 t	22.5 t
2	30.1 t	30.1 t	30.1 t	30.10 t	30.10 t
3	75.3 d	75.3 d	75.4 d	77.4 d	77.4 d
4	45.3 s	45.3 s	45.5 s	42.3 s	42.4 s
5	145.2 s	145.2 s	146.2 s	148.0 s	149.1 s
6	123.2 d	123.2 d	121.1 d	122.5 d	120.3 d
7	68.5 d	68.5 d	78.7 d	68.2 d	78.4 d
8	53.9 d	53.9 d	49.1 d	53.3 d	48.9 d
9	35.0 s	35.0 s	35.1 s	37.3 s	37.4 s
10	39.9 d	39.9 d	40.1 d	41.4 d	41.6 d
11	33.7 t	33.7 t	33.7 t	44.5 t	44.4 t
12	31.5 t	31.5 t	31.4 t	71.9 d	71.8 d
13	47.3 s	47.2 s	47.4 s	52.1 s	52.3 s
14	49.2 s	49.1 s	49.2 s	51.4 s	51.1 s
15	35.6 t	35.7 t	35.8 t	36.1 t	36.2 t
16	28.9 t	28.9 t	28.8 t	25.7 t	25.6 t
17	52.4 d	52.1 d	52.2 d	51.9 d	52.0 d
18	16.0 q	16.0 q	15.9 q	10.6 q	10.6 q
19	29.7 q	29.7 q	29.3 q	29.7 q	29.3 q
20	33.5 d	33.8 d	33.8 d	35.0 d	35.0 d
21	19.0 q	19.3 q	19.3 q	22.1 q	22.1 q
22	41.0 t	45.6 t	45.6 t	39.7 t	39.7 t
23	70.9 d	66.6 d	66.6 d	127.3 d	127.3 d
24	81.6 d	130.5 d	130.5 d	140.0 d	140.1 d
25	146.7 s	133.4 s	133.4 s	71.2 s	71.2 s
26	114.1 t	18.1 q	18.1 q	30.09 q	30.09 q
27	18.0 q	26.0 q	26.0 q	30.0 q	30.0 q
28	23.7 q	23.6 q	23.6 q	28.7 q	28.7 q
29	69.5 t	69.5 t	69.6 t	26.1 q	26.0 q
30	18.6 q	18.6 q	18.8 q	18.5 q	18.5 q
7-OCH$_3$			56.5 q		56.4 q

and the double bond. In this way, HMBC correlations between C-3 (δ_c 75.3) and the signals of the diastereotopic protons at δ_H 3.65 and δ_H 3.95 and Me-28 (δ_H 0.96) and between C-29 (δ_C 69.5) and Me-28 and H-3 placed the OH groups at C-29 and C-3. The OH group at the allylic carbon (C-7) was supported by the correlations between the olefinic carbons C-5 (δ_C 145.2) and C-6 (δ_C 123.2) and the oxymethine proton H-7 (δ_H 3.94). The presence of a diol system at C-23 and C-24 was indicated by the ^{1}H−^{1}H COSY

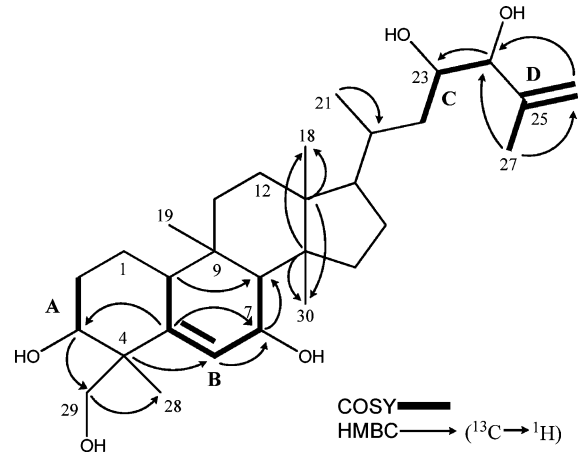


Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of compound **1**.

coupling between H-23 (δ_H 3.63) and H-24 (δ_H 3.74) and was supported by the heteronuclear HMBC correlations observed between C-24 (δ_C 81.6) and H-23 and between C-26 (δ_C 114.1) and C-27 (δ_C 18.0) and H-24. Furthermore, long-range correlations between C-27 and the olefinic protons were also observed. The relative configuration of the tetracyclic system of **1** was determined using a NOESY experiment (Figure 2), taking into account cucurbitacins biogenesis,[21] and by comparison of the coupling constant pattern with that reported in the literature for similar compounds. The cross-peaks observed between H-10/Me-28, Me-28/H-3, and H-7/Me-30 supported a β-orientation of the CH_2OH at C-4 and the OH groups at C-3 and C-7. Moreover, NOE correlations between Me-19/H-8 and H-8/Me-18 corroborated the β-orientation of these protons. In the side chain, the configuration at C-23 and C-24 was deduced as *R,R* by comparing the coupling constants of H-23 (J = 1.86, 7.0, 10.8 Hz) and H-24 (J = 7.0 Hz) of compound **1** with those reported in the literature for a cycloartenol derivative with the same side chain [H-23 (J = 1.7, 6.5, 10.7 Hz) and H-24 (J = 6.5 Hz)].[22] Similar values were also reported for alisol derivatives.[23,24] In spite of the flexibility of the

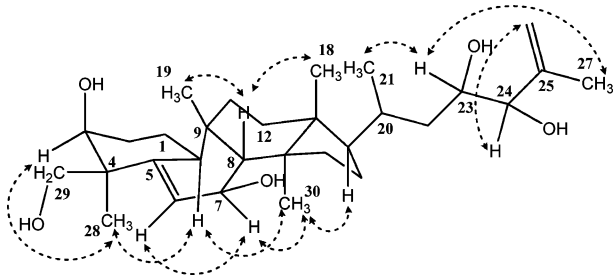


Figure 2. Key NOESY correlations of compound **1**.

side chain, the NOESY spectrum showed strong correlations between Me-21/H-23, H-24/H-26, and H-23/Me-27, which corroborated the *R* configuration of both H-23 and H-24. The energy minimization of the 3D structure of compound **1** was calculated for the four possible configurations, and the *R,R* model showed good agreement with the experimental data (see Supporting Information), suggesting a preferred conformation for the side chain in spite of its possible free rotation. The minimization was carried out with the MMFF99x force field and a root-mean-square gradient at 0.00001, using MOE (Molecular Operating Environment).[25] The pictures of the referenced models of **1** were visualized by using PyMOL.[26] Thus, **1** was determined to be cucurbita-5,25-diene-3β,7β,23(*R*),24(*R*),29-pentaol. To the best of our knowledge, this is the first occurrence of a cucurbitane triterpenoid with a 23,24-diol system coupled to an exocyclic double bond.

Compound **2**, named balsaminol A, had the molecular formula $C_{30}H_{50}O_4$ (ESI-TOF-HRMS), indicating six double-bond equivalents. Comparison of the NMR data of **2** with those of **1** showed that both compounds shared the same triterpenic nucleus, differing only in the side chains. The ^{1}H and ^{13}C NMR spectra of **2** supported the substructure for the side chain as $-CH(CH_3)CH_2CH(OH)CH{=}C(CH_3)_2$, which was corroborated by HMBC correlations observed between C-26 (δ_C 18.1) and C-27 (δ_C 26.0) and the olefinic proton at δ_H 5.16. The configuration at C-23 was assigned as *R*, by comparison of the ^{13}C NMR data of the side chain carbons of **2** with those reported for the lanostane derivative 23(*R*)-3-oxolanosta-8,24-dien-23-ol, the structure of which was determined by X-ray crystallography, and for a cycloartane with the 23(*S*) configuration.[27,28] This assignment was corroborated by a strong NOE effect observed, in the NOESY spectrum, between Me-21 and the proton H-23. The experimental data were supported by the energy minimization of the 3D structure of compound **2** (see Supporting Information).[25,26] Consequently, compound **2** was determined to be cucurbita-5,24-diene-3β,7β,23(*R*),29-tetraol.

Compound **3**, named balsaminol B, gave a pseudomolecular ion peak at *m/z* 511.3757 (calcd for $C_{31}H_{52}O_4Na$, 511.3758) $[M + Na]^+$ in its ESI-TOF-HRMS, corresponding to the molecular formula $C_{31}H_{52}O_4$. The ^{1}H NMR and ^{13}C NMR data were quite similar to those of compound **2**, except for the signals of ring B (see Tables 1 and 2), and indicated that the only difference was the presence of an OCH_3 group at C-7 instead of a hydroxyl group. This was indicated by the OCH_3 singlet at δ_H 3.33 and the corresponding ^{13}C NMR resonance at δ_C 56.5. Its placement at C-7 was corroborated by the heteronuclear $^2J_{C-H}$ correlation of C-7 with H-8 and $^3J_{C-H}$ correlation between C-7 and OMe. The β-orientation of the 7-methoxyl group was assigned by the NOE of H-7 (δ_H 3.49) and the OCH_3 (δ_H 3.33) with the biogenetically α-oriented Me-30 (δ_H 0.77) and β-oriented H-8 (δ_H 2.06), respectively. The structure of **3** was thus determined to be 7β-methoxycucurbita-5,24-diene-3β,23(*R*),29-triol.

Compound **4**, named cucurbalsaminol A, had the molecular formula $C_{30}H_{50}O_4$ (ESI-TOF-HRMS). The IR absorption band at 3447 cm^{-1} indicated OH functions. The ^{1}H NMR spectrum showed resonances for seven tertiary methyls as singlets at δ_H 0.74, 0.89, 1.01, 1.08, 1.18 (3H each), and 1.25 (2 × 3 H), one secondary

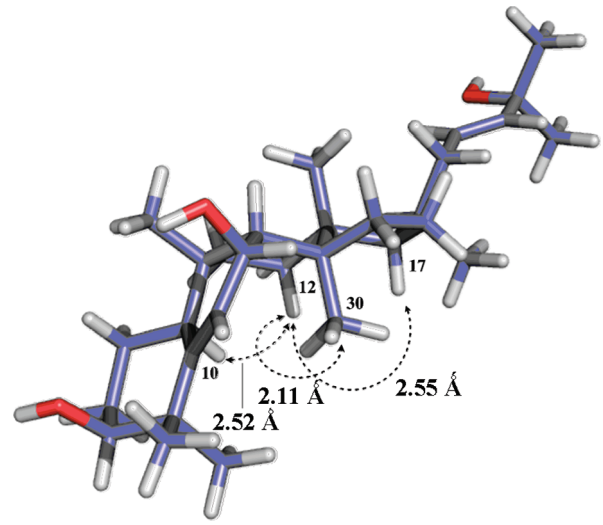


Figure 3. Energy-minimized 3D structure of compound **4**.

methyl as a doublet at δ_H 1.02 (J = 6.8 Hz), and three oxygenated methine protons [δ_H 3.49 (br s), 3.86 (dd, J = 5.0, 11.4 Hz), and 3.94 (d, J = 4.9 Hz)]. Protons of a trisubstituted double bond [δ_H 5.74 (1H, d, J = 4.5 Hz)] and a disubstituted double bond [δ_H 5.58 (m, 2H)] were also observed. The ^{13}C NMR spectrum of **4** revealed 30 carbon signals, which were assigned by a DEPT experiment as eight methyls, six methylenes, 10 methines (including three oxygenated and three vinylic), and six quaternary carbons (one oxygenated and one sp^2). When comparing the ^{1}H and ^{13}C NMR data of the tetracyclic skeleton of **4** with those of compounds **1** and **3**, the paramagnetic effects observed at C-3, C-5, and C-28 ($\Delta\delta_C \cong +2.3, +2.4, +5.0$ ppm, γ-carbons) and the diamagnetic effect at C-4 ($\Delta\delta_C \cong -2.4$ ppm, β-carbon) suggested that the OH group at C-29 was absent in compound **4**. This feature was corroborated by the presence of an additional singlet (δ_H 1.18) in the aliphatic region assigned to Me-29. An unusual OH group at C-12 was indicated by the marked downfield shift at C-12 ($\Delta\delta_C \cong +40$ ppm, α-carbon). Other significant differences were observed, mainly the carbons of ring C and Me-18 (see Tables 1 and 2). The HMBC spectrum corroborated the presence of an OH group at C-12 by $^3J_{C-H}$ couplings observed between C-18 and H-12, and between C-10 and H-11, and the $^2J_{C-H}$ correlations between C-11, C-13, and H-12. The presence of a tertiary OH group at C-25 was supported by HMBC correlations observed between C-25 and the olefinic protons H-23 and H-24, and by Me-26 and Me-27. The relative configuration at C-12 was deduced by significant NOE correlations observed in the NOESY spectrum, which also allowed determination of the relative configuration of the remaining stereocenters as indicated. NOE effects between H-12/H-10 (δ_H 2.30), H-12/Me-30 (δ_H 0.74), and H12/H-17, together with coupling constants, indicated an equatorial β-oriented OH group. Furthermore, taking into account that a significant NOE can usually be detected if the distance between the dipolar-coupled protons is less than 3.5 Å, the calculated conformation[25,26] of compound **4** agreed well with the above-mentioned spectroscopic results (Figure 3). The configuration of the disubstituted double bond at C-23 could not be determined by the vicinal coupling constant values of the olefinic signals, due to overlapping. However, comparison of the ^{13}C NMR chemical shifts of the side chain of **4** with those of both *E*/*Z*-isomers of cycloart-23-ene-3,25-diol[29] allowed assignment of the *E* geometry to the double bond. Therefore, compound **4** was concluded to be cucurbita-5,23(*E*)-diene-3β,7β,12β,25-tetraol.

Compound **5**, named cucurbalsaminol B, had the molecular formula $C_{31}H_{52}O_4$ (HREIMS). The EIMS data of **5** suggested the presence of an OCH_3 group and three OH groups. Comparison of the NMR data of **5** with those of compound **4** revealed that the two compounds were identical except for the signals corresponding

to the proton geminal to the OH group at C-7, which was replaced by an OCH_3 group in **5**. This was supported by the upfield shift of H-7 (δ_H 3.49) when compared with that of compound **4**, and by the HMBC correlation between the OCH_3 group and H-7. Therefore, compound **5** was determined to be 7β-methoxycucurbita-5,23(*E*)-diene-3β,12β,25-triol.

Compounds **6** and **7** were identified on the basis of their spectroscopic data, which were in good agreement with those described in the literature for those compounds: cucurbita-5,23(*E*)-diene-3β,7β,25-triol[19] and karavilagenin E,[20] respectively.

The cytotoxicity of compounds **1**−**4**, **6**, and **7** was evaluated against a breast cancer cell line (MCF-7). The results are shown in Table S1 (Supporting Information). All of the compounds, except **4**, had IC_{50} values ranging from 30.7 to 55.3 μM.

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. IR spectra were determined on a FTIR Nicolet Impact 400, and NMR spectra recorded on a Bruker ARX-400 NMR spectrometer (^{1}H 400 MHz; ^{13}C 100.61 MHz) and Bruker Avance DRX-500 (^{1}H 500 MHz), using CD_3OD as solvent. ESIMS were taken on a Micromass Quattro micro API and ESI-TOF-HRMS on a Bruker-Microtof ESI-TOF (Biotof II Model, Bruker). EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Column chromatography (CC) was carried out on SiO_2 (Merck 9385). TLC was performed on precoated SiO_2 F254 plates (Merck 5554 and 5744) and visualized under UV light and by spraying with sulfuric acid−MeOH (1:1) followed by heating. HPLC was carried out on a Merck-Hitachi instrument, with UV detection (210 nm), using a Merck LiChrospher 100 RP-18 (10 μm, 250 × 10 mm) column.

Plant Material. The aerial parts of *Momordica balsamina* were collected in Gaza, Mozambique, in August 2006. The plant material was identified by the botanist Dr. Silva Mulhovo, and a voucher specimen (30 SM) has been deposited at the herbarium (LMA) of Instituto de Investigação Agronómica, Mozambique.

Extraction and Isolation. Dried aerial parts of *M. balsamina* (1.2 kg) were mechanically powdered and exhaustively extracted with MeOH (11 × 8 L) at room temperature. The MeOH extract was evaporated (under vacuum, 40 °C) to afford a residue (280 g), which was suspended in H_2O (1 L) and extracted with EtOAc (9 × 0.5 L). The EtOAc residue (85 g) was suspended in MeOH−H_2O (9:1; 1 L) and extracted with *n*-hexane (5 × 0.5 L) for removal of waxy material. The remaining extract was evaporated under vacuum (40 °C), yielding a residue (45 g) that was chromatographed over silica gel (1 kg) using mixtures of *n*-hexane−EtOAc (1:0 to 0:1) and EtOAc−MeOH (19:1 to 0:1) as eluents. According to differences in composition as indicated by TLC, six crude fractions were obtained (fractions 1−6). Fraction 2 (2.1 g), eluted with a mixture of *n*-hexane−EtOAc (1:1), was chromatographed using mixtures of *n*-hexane−EtOAc. A subfraction (372 mg) was separated by repeated CC with mixtures of CH_2Cl_2−acetone. A final purification was carried out by HPLC to afford compounds **6** (8 mg, 210 nm, MeOH−H_2O, 41:9, 4 mL/min, t_R = 24 min) and **7** (9 mg, ACN−H_2O, 22:3, 4 mL/min, t_R = 26 min). The residue (5.3 g) of fraction 3 (*n*-hexane−EtOAc, 1:1 to 0:1) was subjected to a silica gel CC with mixtures of *n*-hexane−EtOAc to give several fractions. The residue (408 mg) eluted with *n*-hexane−EtOAc (1:3 to 3:17) was successively chromatographed using gradients of CH_2Cl_2−acetone and further purified by HPLC to yield 11 mg of compound **5** (220 nm, MeOH−H_2O, 17:3, 5 mL/min, t_R = 8 min). Fraction 4 (3.25 g), eluted with mixtures of *n*-hexane−EtOAc (1:19 to 0:1), was chromatographed over SiO_2 with mixtures of *n*-hexane−EtOAc to give seven fractions. A subfraction (1.0 g), eluted with *n*-hexane−EtOAc (1:3 to 1:19) was successively rechromatographed by CC using gradients of *n*-hexane−EtOAc and CH_2Cl_2−MeOH and further purified by HPLC (210 nm, MeOH−H_2O, 4:1, 5 mL/min, t_R = 23 min), yielding 12 mg of compound **3**. Another subfraction (1.3 g), eluted with EtOAc−MeOH (1:0 to 9:1), was also successively rechromatographed by CC, using mixtures of CH_2Cl_2−MeOH, and further purified by HPLC (210 nm, MeOH−H_2O, 3:1, 4 mL/min, t_R = 16 min) to afford **4** (32 mg).

The residue (2.8 g) of fraction 5 (EtOAc−MeOH, 99:1 to 93:7) was chromatographed on SiO_2 with mixtures of *n*-hexane−EtOAc (1:1 to 0:1) and EtOAc−MeOH (49:1 to 0:1), giving four fractions. Compound **2** (25 mg) was obtained from a subfraction eluted with *n*-hexane−EtOAc (3:17 to 1:19), after repeated chromatography with CH_2Cl_2−MeOH and purification by HPLC (MeOH−H_2O, 3:1, 5 mL/min, t_R = 40 min). Compound **1** (10 mg) was also obtained by HPLC (210 nm, MeOH−H_2O, 7:3, 5 mL/min, t_R = 28 min), after CC, with the same solvent, of a subfraction eluted with mixtures of increasing polarity of *n*-hexane−EtOAc (1:4 to 1:9).

Balsaminapentaol A, Cucurbita-5,25-diene-3β,7β,23(*R*),24(*R*),29-pentaol (1): amorphous, white powder; $[\alpha]_D^{26}$ +85 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3408, 1651, 1457, 1408, 1380, 1078, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z*: 513 $[M + Na]^+$; ESI-TOF-HRMS *m/z* 513.3553 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3550).

Balsaminol A, Cucurbita-5,24-diene-3β,7β,23(*R*),29-tetraol (2): amorphous, white powder; $[\alpha]_D^{26}$ +93 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3357, 1450, 1360, 1315, 1020, 944, 833, 733 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 497 $[M + Na]^+$; ESI-TOF-HRMS *m/z* 497.3601 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3601).

Balsaminol B, 7β-Methoxycucurbita-5,24-diene-3β,23(*R*),29-triol (3): amorphous, white powder; $[\alpha]_D^{26}$ +90 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3398, 1455, 1382, 1182, 1078, 1030, 936 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 511 $[M + Na]^+$; ESI-TOF-HRMS *m/z* 511.3757 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_4Na$, 511.3758).

Cucurbalsaminol A, Cucurbita-5,23(*E*)-diene-3β,7β,12β,25-tetraol (4): amorphous, white powder; $[\alpha]_D^{26}$ + 89 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3447, 1459, 1380, 1151, 1081, 1020, 980 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 497 $[M + Na]^+$, 369 $[M + Na - \text{side chain}]^+$; ESI-TOF-HRMS *m/z* 497.3601 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_3Na$, 497.3601).

Cucurbalsaminol B, 7β-Methoxycucurbita-5,23(*E*)-diene-3β,12β,25-triol (5): amorphous, white powder; $[\alpha]_D^{26}$ +96 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3388, 1457, 1381, 1081, 1024, 975, 940 cm^{-1}; H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 488 $[M]^+$ (28), 470 $[M - H_2O]^+$ (100), 456 (16), 452 $[M - 2 \times H_2O]^+$ (7), 420 $[M - 2 \times H_2O - OCH_3]^+$ (2), 402 $[M - 3 \times H_2O - OCH_3]^+$ (1), 388 $[M - 3 \times H_2O - OCH_3 - Me]^+$ (1), 344 $[M - \text{side chain} - CH_3]$ (10), 312 $[M - \text{side chain} - CH_3 - OCH_3]^+$ (4), 270 (2); 223 (24), 203 (18), 182 (23), 173 (11), 164 (26), 149 (37), 123 (20), 109 (50), 81 (17); HREIMS *m/z*: 488.3853 $[M]^+$ (calcd for $C_{31}H_{52}O_4$ $[M]^+$, 488.3866).

Cell Culture. Human breast cancer MCF-7 cell line was cultured in RPMI 1640 medium supplemented with 10% heat inactivated horse serum, L-glutamine (2 mM), and antibiotics, in a humidified atmosphere of 5% CO_2 at 37 °C.

Assay for Cytotoxicity. The effects of increasing concentrations of the compounds on cell growth were tested in 96-well flat-bottomed microtiter plates. The compounds were diluted in a volume of 50 μL of medium. Then, 2 × 10^4 cells in 0.1 mL of medium were added to each well, with the exception of the medium control wells. The culture plates were further incubated at 37 °C for 24 h. At the end of the incubation period, 15 μL of MTT (thiazolyl blue, Sigma, St Louis, MO) solution (from a 5 mg/mL stock) was added to each well. After incubation at 37 °C for 4 h, 100 μL of sodium dodecyl sulfate (SDS) (Sigma, St Louis, MO) solution (10%) was measured into each well, and the plates were further incubated at 37 °C overnight. The cell growth was determined by measuring the optical density (OD) at 550 nm (ref 630 nm) with a Dynatech MRX vertical beam ELISA reader. Inhibition of cell growth (as a percentage) was determined according to the formula:

$$100 - \left[\frac{OD_{sample} - OD_{mediumcontrol}}{OD_{cellconrol} - OD_{mediumcontrol}}\right] \times 100$$

Acknowledgment. This study was supported by FCT, Portugal (BD/22321/2005). The authors thank Dr. T. Vasconcelos, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal, for the taxonomic work on the plant material, and also Dr. C. Arruda from the Portuguese Embassy in Mozambique, as well as the Portuguese Office of International Affairs for plant transport. We also are grateful to A. S. Newton for her help with 3D structures.

Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**5**, energy-minimized 3D structures of compounds **1** and **2**, and Table S1 are available free of charge via the Internet at http://pubs.acs.org.

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NP900457U