Studies on the Cytotoxicity of Cucurbitacins Isolated from *Cayaponia racemosa* (Cucurbitaceae)

Ivana Nogueira Fernandes Dantas[a], Gardenia Carmen Militão Gadelha[a], Davina Camelo Chaves[b], Francisco José Queiroz Monte[b], Cláudia Pessoa[a], Manoel Odorico de Moraes[a], and Letícia Veras Costa-Lotufo[a,*]

[a] Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, P.O. Box 3157, 60430-270 Fortaleza, Ceará, Brazil. Fax: # 55 85 4009 8333. E-mail: lvcosta@secrel.com.br

[b] Department of Organic and Inorganic Chemistry, Federal University of Ceará, P.O. Box 12200, 60021-940 Fortaleza, Ceará, Brazil

* Author for correspondence and reprint requests

Z. Naturforsch. **61c**, 643–646 (2006); received March 6/May 3, 2006

The cytotoxic potential of three cucurbitacins, 2,3,16,20(*R*),25-pentahydroxy-11,22-dioxocucurbita-5-en (cucurbitacin P, **1**), 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5-en (**2**) and 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5,23(*E*)-diene (deacetylpicracin, **3**), obtained from *Cayaponia racemosa* was evaluated as their ability to induce brine shrimp lethality, to inhibit the development of sea urchin eggs and tumor cell proliferation, and to lysis mouse erythrocytes. Compounds **1** and **2** were highly toxic with LC_{50} of (29.6 ± 9.1) (56.8) and (38.8 ± 3.0) (76.6) μg/mL (μM), respectively, while compound **3** was not effective at the tested concentrations. All tested compounds possessed an inhibitory effect on the proliferation of tumor cell lines, compound **1** being the most active, followed by **2** and **3**. Nevertheless, no hemolytic activity or inhibition of the development of sea urchin eggs was observed for these compounds.

Key words: Cayaponia racemosa, Cucurbitacins, Cytotoxic Activity

Introduction

Cucurbitacins are a special group of triterpenoids having a cucurbitane skeleton with a wide range of biological activities associated with their chemical structural complexity (Chen *et al*., 2005). They are characteristic (but not exclusive) constituents of members of the Cucurbitaceae family. The most relevant biological roles are their strong antifeedant activity towards a number of insect species (Sachdev-Gupta *et al*., 1993) and insecticidal activity acting at the ecdysteroid receptor (Dinan *et al*., 1997). Besides, they also possess remarkable pharmacological effects, like inhibition of cell adhesion (Musza *et al*., 1994) and of T-lymphocyte proliferation (Smit *et al*., 2000), anti-inflammatory (Peters *et al*., 1997; Jayaprakasam *et al*., 2003), analgesic (Peters *et al*., 1997), immunomodulatory (Attard *et al*., 2005) and anti-angiogenic activities (Duncan and Duncan, 1997). Previous reports have attributed to the cucurbitacins *in vitro* and *in vivo* anticancer activities (Fang *et al*., 1984; Duncan *et al*., 1996; Clericuzio *et al*., 2004).

Cayaponia racemosa Cogn. (Cucurbitaceae) is a climber plant commonly found in northeastern Brazil, where it is popularly known as "guardião" (Barroso, 1978). The seeds of *C. racemosa* are used in Brazilian folk medicine in dermatology and to treat epilepsy, amenorrhea and constipation (Braga, 1976). In the course of screening natural products as new anticancer agents, we have evaluated the cytotoxic activity of the cucurbitacins 2,3,16,20(*R*),25-pentahydroxy-11,22-dioxocucurbita-5-en (cucurbitacin P, **1**), 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5-en (**2**) and 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5,23(*E*)-diene (deacetylpicracin, **3**) isolated from *C. racemosa*. It is worthwhile to mention that this is the first report on the isolation of these cucurbitacins from *C. racemosa*.

The cytotoxicity of these cucurbitacins was evaluated as their ability to induce brine shrimp lethality, to inhibit the development of sea urchin eggs and tumor cell proliferation, and to lysis mouse erythrocytes.

0939–5075/2006/0900–0643 $ 06.00

Material and Methods

Plant material

The plant material was collected in Palmácia, Ceará, Brazil. A voucher specimen (# 30532) was deposited at the Herbário Prisco Bezerra, Federal University of Ceará, Brazil. The air-dried, powdered fruits (0.91 kg) of *C. racemosa* were extracted exhaustively with EtOH (3 L) at room temperature. After removal of the solvent under reduced pressure, the dark green residue (27.8 g) was fractionated by silica gel (Kieselgel 60 H) column chromatography using hexane, $CHCl_3$, EtOAc and MeOH as eluents. The EtOAc fraction (8.34 g) contained a mixture of cucurbitacins. A portion (700.0 mg) of this fraction was subjected to reversed-phase HPLC on a Hibar RP-18 column (250 × 4 mm) eluted with MeOH/H_2O (6:4), to yield 2,3,16,20(*R*),25-pentahydroxy-11,22-dioxocucurbita-5-en (**1**) [280.0 mg, $[\alpha]_D^{25}$ + 305.6° (*c* 0.0055, MeOH), m.p. 141.5–144 °C] and 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5-en (**2**) [325.0 mg, $[\alpha]_D^{25}$ – 687.6° (*c* 0.43, C_5H_5N), m.p. 141–144 °C]. Another portion (60.0 mg) of the EtOAc fraction was purified by preparative layer chromatography [silica gel, $CHCl_3$/MeOH (9.5:0.5) developed three times] and gave 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5, 23(*E*)-diene (**3**) [30.0 mg, $[\alpha]_D^{25}$ – 320,7° (*c* 0.55, MeOH), m.p. 182.4–185 °C]. Structural elucidations of **1**, **2** and **3** were based mainly on spectroscopic [infrared (IV)] and ^1H and ^{13}C NMR analysis including special pulses sequences (one and two dimensional NMR such as DEPT 135°, $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, $^1H-^{13}C$ HMBC and $^1H-^1H$ NOESY) and comparison with data from literature (Stuppner *et al.*, 1981; El-Fattah, 1994).

Brine shrimp lethality assay

The brine shrimp lethality assay was performed in 24-multiwell plates. Ten nauplii were incubated for 24 h with the compounds (1 to 100 μg/mL) in multiwell plates. Survivors were counted after incubation and the percentage of deaths at each concentration and control (seawater) were determined (Meyer *et al.*, 1982).

In vitro cytotoxic activity

The cytotoxicity of the compounds was investigated against HL-60 (human leukemia), CEM (human leukemia), HCT-8 (human colon cancer), MCF-7 (human breast cancer) and B-16 (murine melanoma) tumor cell lines (National Cancer Institute, Bethesda, MD, USA). Cells were plated in 96-multiwell plates. The compounds (0.39 to 25 μg/mL) dissolved in 10% DMSO were added and the cells were incubated for 72 h. Doxorubicin (0.01 to 0.58 μg/mL) was used as positive control. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye MTT to a purple formazan product (Mosmann, 1983).

Antimitotic activity on sea urchin eggs

Adult sea urchins (*Lytechinus variegatus*) were collected at Lagoinha beach, on the northeastern Brazilian coast. The gamete elimination was induced by KCl and for *in vitro* fertilization the sperm suspension was added to the egg solution. The assay was carried out in 24-multiwell plates. The compounds were added immediately after fecundation to get concentrations of 1, 3, 10, 30 and 100 μg/mL. Doxorubicin was used as positive control (0.0058 to 58.0 μg/mL). Aliquots of 200 μL were fixed in the same volume of formaldehyde to obtain first and third cleavage and blastulae stages (Jimenez *et al.*, 2003).

Hemolytic activity assay

The hemolytic assay was performed as described by Jimenez *et al.* (2003). The compounds were tested at concentrations ranging from 2.3 to 300 μg/mL. Triton X-100 (0.1%) was used to obtain 100% hemolysis. After incubation, the liberated hemoglobin was measured spectroscopically at 540 nm.

Statistical analysis

Data are presented as means ± S.E.M. The IC_{50} values and their confidence intervals were obtained by nonlinear regression using the GraphPad Prism Software (Intuitive Software for Science, San Diego, CA). LC_{50} values for brine shrimp assay were obtained from 24-h counts using the probit analysis method described by Litchfield and Wilcoxon (1949).

Results and Discussion

In the brine shrimp lethality assay, compounds **1** and **2** were highly toxic with LC_{50} of (29.6 ± 9.1) (56.8) and (38.8 ± 3.0) (76.6) μg/mL (μM), respectively, while compound **3** was not effective at the tested concentrations. According to several au-

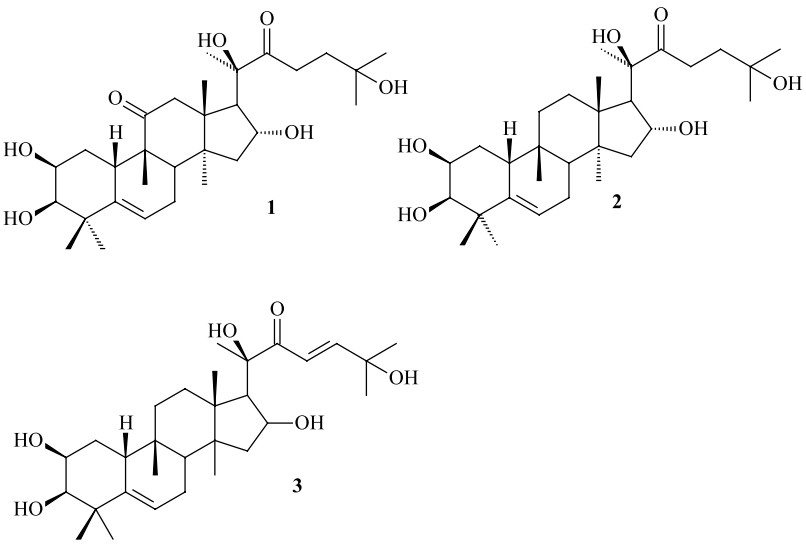

Fig. 1. Chemical structures of the cucurbitacins 2,3,16,20(*R*),25-pentahydroxy-11,22-dioxocucurbita-5-en (cucurbitacin P, **1**), 2,3,16,20(*R*),25-pentahydroxy-22-oxocucur-bita-5-en (**2**) and 2,3,16,20(*R*),25-pentahydroxy-22-oxocucurbita-5,23(*E*)-diene (deacetylpicracin, **3**) isolated from *Cayaponia racemosa.*

thors, this assay presents a good relationship with assays to detect antitumor compounds in terrestrial plants extracts (Meyer *et al*., 1982). In the present paper, this correlation was not quite evident, since all tested compounds possessed inhibitory effects on the proliferation of tumor cell lines, compound **1** being the most active, followed by **2** and **3** (Fig. 1, Table I). Previous studies reported the *in vitro* cytotoxic activity of some structurally related cucurbitacins (Jayaprakasam *et al*., 2003; Clericuzio *et al*., 2004). According to Duncan and Duncan (1997), the cucurbitacins E, B, I, D, Q, K (but not P) presented cytotoxic activity against the PC-3 (prostate carcinoma) cell line. Thus, the authors postulated that the lack of a double bond in the side chain between C-23 and C-24 and hydroxylation of C-23 would decrease the cytotoxicity. The previous work of Fang *et al*. (1984) reinforced the importance of these structural requirements, in view of the fact that cucurbitacin F presented cytotoxicity against P-388 e KB cell lines, while dihydrocucurbitacin F and hexanorcucurbitacin F were not effective. Nevertheless, the data exhibited here showed exactly the opposite, since the strongest inhibitory activity would be related to cucurbitacin P (**1**), a compound that lacks the double bond in the side chain, while deacetylpicracin (**3**), which possesses the double bond in the side chain, was the least active. Another difference between the three cucurbitacins isolated from *C. racemosa*, that could justify the highest activity, is the presence of a carbonyl group at C-11 of cucurbitacin P (**1**). Clericuzio *et al*. (2004) demonstrated the growth inhibitory activity of cucurbitacin B, which possesses a carbonyl group at C-11, in contrast to two other cucurbitane triterpenoids studied, leucopaxillones A and B, which were less effective.

Compound	IC_{50}, CI 95% [μg/mL (μM)]				
	HL-60	CEM	B-16	HCT-8	MCF-7
Doxorubicin	0.02 (0.03) 0.01–0.02	0.02 (0.03) 0.01–0.02	0.03 (0.05) 0.02–0.04	0.04 (0.07) 0.03–0.05	0.20 (0.34) 0.17– 0.24
1	0.64 (1.20) 0.49–0.85	0.80 (1.50) 0.72–0.88	0.99 (1.86) 0.91–1.07	0.96 (1.80) 0.66–1.39	1.71 (3.20) 1.48– 1.96
2	1.35 (2.70) 1.07–1.70	1.89 (3.78) 1.69–2.12	2.22 (4.44) 1.91–2.58	1.68 (3.36) 1.52–1.84	3.58 (7.16) 3.23– 3.98
3	2.35 (4.60) 1.89–2.92	2.31 (4.52) 2.04–2.61	4.97 (9.70) 3.40–7.26	2.92 (5.70) 2.04–4.17	7.68 (15.29) 5.73–10.28

Table I. Cytotoxic activity of the cucurbitacins isolated from *Cayaponia racemosa* on tumor cell lines determined by the MTT assay.

The IC_{50} values and their 95% confidence interval (CI 95%) were obtained by nonlinear regression. Doxorubicin was used as positive control.

The sea urchin eggs assay provides a useful manner to evaluate the disturbance of a compound in the phases of the cell cycle. However, present results indicate that none of the tested compounds inhibited the sea urchin development.

The similar chemical structures and very high biological activity of cucurbitacins and steroids suggest a similar mechanism of biological action of both groups of compounds (Duncan and Duncan, 1997). Nevertheless, no hemolytic activity was observed in the hemolytic assay using mice erythrocytes.

In conclusion, the cucurbitacins from *Cayaponia racemosa* exhibited potent anticancer activity. The results so far shown suggest that the presence of a carbonyl group at C-11 and the absence of the double bond between C-23 and C-24 are correlated to the increasing activity. Besides, the activity observed was very selective to cancer cells, since no inhibition was observed in the sea urchin assay. At last, the cytotoxic activity was not linked to induction of membrane disruption.

Acknowledgements

We wish to thank CNPq, FUNCAP, BNB/FUNDECI, FINEP and CAPES for financial support and National Cancer Institute (NCI-USA) for the donation of the tumor cell lines. The authors also thank Silvana França for technical assistance.

Attard E., Brincat M. P., and Cuschieri A. (2005), Immunomodulatory activity of cucurbitacin E isolated from *Ecballium elaterium*. Fitoterapia **76**, 439–441.

Barroso M. G. (1978), Sistemática de Angiospermas do Brasil. Imprensa Universitária, Viçosa, Minas Gerais, Brazil, pp. 197–198.

Braga R. (1976), Plantas do Nordeste, Especialmente do Ceará, 3ª Ed., Vol. XLII, 98. Imprensa Universitária, Fortaleza, Ceará, Brazil.

Chen J. C., Chiu M. H., Nie R. L., Cordell G. A., and Qiu S.X. (2005), Cucurbitacins and cucurbitane glycosides: structures and biological activities. Nat. Prod. Rep. **22**, 386–399.

Clericuzio M., Mella M., Vita-Finzi P., Zema M., and Vidari G. (2004), Cucurbitane triterpenoids from *Leucopaxillus gentianeus*. J. Nat. Prod. **67**, 1823–1828.

Dinan L., Whiting P., Girault J., Lafont R., Dhadialla T. S., Cress D. E., Mugat B., Antoniewski C., and Lepesant J. (1997), Cucurbitacins are insect steroid hormone antagonists acting at the ecdysteroid receptor. Biochem. J. **327**, 643–650.

Duncan M. D. and Duncan K. L. K. (1997), Cucurbitacin E targets proliferating endothelia. J. Surg. Res. **69**, 55–60.

Duncan K. L. K., Duncan M. D., Alley M. C., and Sausville E. A. (1996), Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochem. Pharmacol. **52**, 1553–1560.

El-Fattah H. A. (1994), Structure revision of cucurbitacin Q_1. Phytochemistry **36**, 159–161.

Fang X., Phoebe Jr. C. H., Pezzuto J. M., Fong H. H. S., Farnsworth N. R., Yellin B., and Hecht S. M. (1984), Plant anticancer agents, XXXIV. Cucurbitacins from Elaeocarpus dolichostylus. J. Nat. Prod. **47**, 988–993.

Jayaprakasam B., Seeram N. P., and Nair M. G. (2003), Anticancer and antiinflammatory activities of cucurbitacins from *Cucurbita andreana*. Cancer Lett. **189**, 11–16.

Jimenez P. C., Fortier S. C., Lotufo T. M. C., Pessoa C., Moraes M. E. A., Moraes M. O., and Costa-Lotufo L. V. (2003), Biological activity in extracts of ascidians (Tunicata, Ascidiacea) from the northeastern Brazilian coast. J. Exp. Mar. Biol. Ecol. **287**, 93–101.

Litchfield J. T. and Wilcoxon F. (1949), A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. **95**, 99–113.

Meyer B. N., Ferrigni N. R., Putnam J. E., Jacobsen L. B., Nichols D. E., and McLaughlin J. L. (1982), Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. **45**, 31–34.

Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65**, 55–63.

Musza L. L., Speight P., McElhiney S., Barrow C. J., Gillum A. M., Cooper R., and Killar L. M. (1994), Cucurbitacins, cell adhesion inhibitors from *Conobea scoparioides*. J. Nat. Prod. **57**, 1498–1502.

Peters R. R., Farias M. R., and Ribeiro-Do-Valle R. M. (1997), Anti-inflammatory and analgesic effects of cucurbitacins from *Wilbrandia ebracteata*. Planta Med. **63**, 525–528.

Sachdev-Gupta K., Radke C. D., and Renwick A. A. (1993), Antifeedant activity of cucurbitacins from *Iberis amara* against larvae of *Pieris rapae*. Phytochemistry **33**, 1385–1388.

Smit H. F., Van Den Berg A. J. J., Kroes B. H., Beukelman C. J., Van Ufford H. C. Q., Van Dijk H., and Labadie J. (2000), Inhibition of T-lymphocyte proliferation by cucurbitacins from *Picrorhiza scrophulariaeflora*. J. Nat. Prod. **63**, 1300–1302.

Stuppner H., Muller E. P., and Wagner H. (1981), Cucurbitacins from *Picrorhiza kurooa*. Phytochemistry **30**, 305–310.