A New Cucurbitacin Glycoside from *Kageneckia oblonga* (Rosaceae)

Orlando Muñoz[a,*], Carla Delporte, Nadine Backhouse, Silvia Erazo, Rosa Negrete, Sergio Maldonado[b], José L. López-Pérez and Arturo San Feliciano[c]

[a] Departamento de Química, Facultad de Ciencias, Universidad de Chile. Casilla 653. Santiago, Chile. Telefax: 56(2) 2713888. E-mail: omunoz@abello.dic.uchile.cl
[b] Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. Casilla 233. Santiago, Chile
[c] Departamento de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca. 37007 Salamanca, España

* Author for correspondence and reprint requests

Z. Naturforsch. **55c,** 141–145 (2000); received October 13/November 19, 1999

Kageneckia oblonga, Rosaceae, Cucurbitacin

A novel cucurbitacin glycoside has been isolated from aerial parts of *Kageneckia oblonga* R. *et* P. and shown to be 3β-(β-D-glucosyloxy)-16α,23α-epoxycucurbita-5,24-dien-11-one. The structure was established by usual spectroscopic and two-dimensional (2D) NMR techniques. This compound has found to be nontoxic when tested *in-vivo* cell culture assays. In previous investigations we reported 23,24-dihydrocucurbitacin F and prunasine. This was the first report on cucurbitacins from the genus *Kageneckia* (Rosaceae).

Introduction

Kageneckia oblonga R. *et* P. commonly known as "huayu", "huayu colorado" or "bollén", is a tall tree (15 m high, 40 cm diam.) with evergreen, coriaceus leaves (Rodriguez *et al.*, 1995); endemic to Central and Southern Chile. The tree grows well in dry or semihumid soils and is found in the Andes Mountains below 1800 m. Stems and leaves of *Kageneckia* have been widely used in traditional medicine, mainly in infusion, as a potion to treat hepatic and kidney disorders and many other ailments (San Martín, 1983; Muñoz *et al.*, 1981). Previous phytochemical investigations reported on the isolation of common triterpenoids (Cassels *et al.*, 1973), prunasin (Fikenscher *et al.*, 1981) and 23,24-dihydrocucurbitacin F (Maldonado *et al.*, 1996a). Cytotoxical studies carried out with its different extracts showed them to be non-cytotoxic when tested on several neoplastic cell lines (Maldonado *et al.*, 1996b). Antiinflammatory, antipyretic and analgesic activities of different extracts have also been previously reported (Maldonado *et al.*, 1996a; Maldonado *et al.*, 1996b; Delporte *et al.*, 1997; Delporte *et al.*, 1998).

In continuation of these investigations and of our search for the pharmacologically active compounds, we report the isolation and structure elucidation of a new cucurbitacin glycoside **1,** as a constituent of *K. oblonga*. Usually, cucurbitacins are known to posses high cytotoxicity and antitumoral properties (Gallily *et al.*, 1992; Gitter *et al.*, 1961). In some cases, as for cucurbitacin B, their antiinflammatory properties have been reported (Yesilada *et al.*, 1988).

Results and Discussion

Repeated chromatography of the MeOH extract of *K. oblonga* followed by crystallization, led to the isolation of a small amount of a new compound **1**, whose structure is being established in this work.

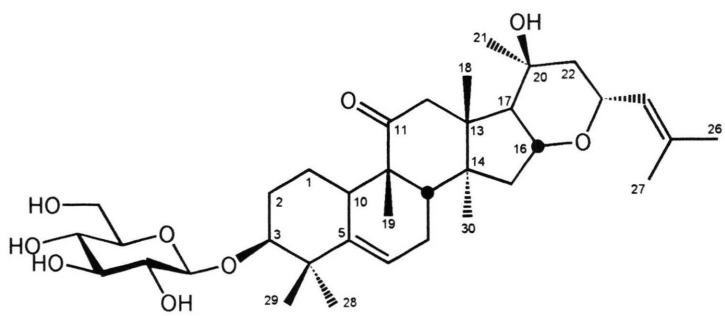
A direct comparison of NMR spectra of **1** with those of 23,24-dihydrocucurbitacin F (El-Fattah, 1994), revealed the structural similarity of both compounds. The main differences observed between them, lied on the presence of additional signals for **1**, in the regions of 2.8–5 ppm (^{1}H) and 61–77 and 100.4 ppm (^{13}C), which revealed the presence of a sugar unit in **1** (Table I). Other structural differences were deduced from a more complete analysis of the spectra. Thus, differences in the side chain were deduced from the presence of two methyl singlets at 1.56 and 1.61 ppm and a broad olefinic doublet at 5.61 ppm in the ^{1}H spectrum, supporting the existence of a 2,2-dimethylvinyl fragment in the structure. On the other hand, some structural aspects present in

0939–5075/2000/0300–0141 $ 06.00

Table I. 1D- and 2D-NMR data for cucurbitacin glucoside 1.

C atom	Type (DEPT)	(ppm)	Attached Hs	Long-range connected Hs
1	CH_2	24.2	1.43 *m*	
2	CH_2	25.8	1.35 *m*	
3	CH-O	81.3	3.12 *m*	1', 2, 28, 29
4	C	41.1	-	6, 28, 29
5	C=	142.2	-	7, 28, 29
6	CH=	117.5	5.61 *d* (5.5)	7
7	CH_2	23.5	1.75 *m*; 2.30 *m*	6, 8
8	CH	41.9	1.80 *m*	19, 30
9	C	48.4	-	19
10	CH	34	2.18 *m*	6, 8, 19
11	CO	112.7	-	12, 19
12	CH_2	48.2	2.16 *d* (14.6); 3.10 *d*(14.6)	18
13	C	47.6	-	8, 12, 30
14	C	47.4	-	12, 17, 18
15	CH_2	39.8	1.20 *m*; 1.70 *m*	30
16	CH-O	75.3	4.22 *dt* (10.4, 3.6)	17
17	CH	54.6	1.75 *d* (10.4)	18, 21
18	CH_2	19.3	0.78 *s*	12, 17
19	CH_3	19.9	0.89 *s*	
20	C-O	70.2	-	21
21	CH_3	28.7	1.13 *s*	22
22	CH_2	48.4	1.15 *s*	21
23	CH-O	72.1	4.40 *dt* (8.4, 3.7)	16, 22
24	CH=	126.3	5.02 *bd* (8.3)	23, 27
25	C=	133	-	23, 27
26	CH_3	25.2	1.61 *s*	24, 27
27	CH_3	18	1.56 *s*	24, 26
28	CH_3	24.5	0.85 *s*	29
29	CH_3	21.4	1.13 *s*	28
30	CH_3	20.6	1.08 *s*	8
1'	CH-O	100.4	4.12 *d* (7.7)	2', 3
2'	CH-O	73.5	2.85 *dd* (9.0, 7.7)	
3'	CH-O	76.9	3.12 *m*	2', 5'
4'	CH-O	70.3	3.01 *m*	
5'	CH-O	76.6	3.01 *m*	6'
6'	CH_2	61.3	3.38 *dd* (11.4, 5.5) 3.65 *dd* (11.4, 1.6)	4'

Solvent: DMSO-*d6*. TMS as internal standard. HMBC experiments were focused for *J* (H/C) = 7 and 3 Hz.



compound 23,24-dihydrocucurbitacin F, such as the Δ^5 double bond (142.2, 117.5 and 5.61 ppm), the carbonyl group at C-11 (212.7 ppm; isolated AB system centered at 2.16 and 3.10 ppm, J = 14.6 Hz: methylene C-12) and the tertiary hydroxyl group at C-20 (70.2 ppm), were also present in the structure of **1**. The overlapping of a number of signals in the ^{1}H NMR spectrum, did not allow to perform the complete analysis of several absorptions important for the unequivocal structural determination; consequently some 2D-NMR experiments (HMQC, COSY, HMBC and ROESY) were run, in two different solvent conditions (DMSO-d_6, and DMSO-d_6+D_2O), in order to achieve a better information about the surroundings of the oxygenated positions within the molecule. The most important NMR data and connectivities detected with the two-dimensional spectra are gathered in Table I.

The results confirm the cucurbitane skeleton for compound **1** and allowed us to assign all the signals in the carbon and proton spectra. The following features are relevant to be pointed out. The signal for the anomeric carbon (100.4 ppm, 1') of the sugar showed long-range correlation with the proton doublet at δ 3.12 ppm, while the carbon atom (81.3 ppm) attached to the latter, was found to correlate with the *gem*-dimethyl signals at 0.85 and 1.13 ppm. It also correlated with a quaternary atom signal at 41.1 ppm, thus indicating that the glycosyl fragment was attached to C-3. Selective assignment of signals for the C-4 methyl groups was based on the ROE effects described below.

The side-chain structure was easily deduced from the analysis of ^{1}H and COSY spectra and confirmed by comparison with data reported for other cucurbitacins (Schenkel *et al.*, 1992) and HMBC data in Table I. The olefinic methyl signals at the end of the chain (18.0 and 25.2 ppm), correlated with the olefinic proton at C-24 (5.02 ppm), which was coupled to the oxygenated methine at position C-23 (4.40/72.1 ppm). The presence of another oxygenated function at C-16 (75.3) was deduced from the correlation with the methine CH-17, whose unequivocal assignment was based on its successive connectivities with signals for protons/carbons at positions HO-C-20 (70.2 ppm) CH_3-21 (1.13/28.7 ppm) and CH_2-22 (1.15/48.4 ppm). The important observation of a long-range correlation between the methine O-CH-23 (72.1 ppm) and H-16 (4.22 ppm) was consistent with the existence of a 16,23-epoxy bridge, closing a tetrahydropyran ring.

The configurations at C-16 and C-23 were deduced from the ROE correlation connecting H-16 and H-23. H-6 signal showed ROE with both methyl groups at C-4 (0.85 and 1.13 ppm), in agreement with its planar equatorial disposition. Furthermore, the axial methyl group (0.85 ppm) showed ROE correlation with H-10, thus confirming the assignment of proton and ^{13}C signals for positions 28 and 20. H-3 also showed ROE correlations with both H-28 and H-29, supporting its equatorial disposition and permitting to assign the 3β-configuration to the glucosyloxy moiety attached at C-3.

The δ value (4.12 ppm) of the anomeric proton of the sugar, its coupling constant (J = 7.7 Hz) and the pattern of absorption for the sugar residue (Table I) confirmed the nature of a β-glucopyranoside (Hatam *et al.*, 1989) for **1**.

Finally, all other correlations mentioned in Table I and the ROE effects observed in two 2D spectra, obtained with different mixing delays between pulses, were in agreement with the structure of 3β-(β-D-glucosyloxy)-16α,23α-epoxycucurbita-5,24-diene-11-one.

In terms of bioactivity, most of the cucurbitacins are known to posses high cytotoxicities and potent antitumoral effects (Gitter *et al.*, 1961; Yesilada *et al.*, 1988). The GEE (Global Ethanolic Extract) showed non specific cytotoxicy (2.5 μg/ml) against all the cells lines. Nevertheless, the new cucurbitacin **1** did not show appreciable cytotoxicity against different cell lines (IC>10 μg/kg) (Table II). Accordingly, previous studies (El-Fattah, 1994) had postulated that the cytotoxic activities of cucurbitacins were due to arrangement of the functional groups at the side chain. The presence of the epoxy 16α,23α in **1**, seems to support this postulate an the absence of cytotoxicity.

Table II. Cytotoxicity activity.

Sample	CI_{50} [μg/ml]		
	P-388	A-549	HT-29
GEE	2.5	2.5	2.5
1	>10	>10	>10
Adriamycin	0.02	0.05	0.11

Experimental

Plant material. Aerial part of *K. oblonga* was collected at Lagunillas, Santiago, Chile, in May 1994, and identified by Dr. Carla Delporte. A voucher specimen is kept at the Herbarium of the Escuela de Química y Farmacia (SQF: 22144a), Universidad de Chile.

General. Column chromatography was run using silica gel 60G (Merck 7734), LH-20 Sephadex (Pharmacia) or Amberlite XAD-2 as non ionic polymeric adsorbent (Aldrich). TLC was performed on silica gel GF254 (Merck 5554) spots were detected under UV light or spraying with anysaldehyde reagent and heating for 5–10 min at 120°. Prep. TLC was performed on 2 mm thick silica/gel F254 plates (Merck 7731). ^{1}H and ^{13}C NMR were recorded in DMSO-d_6 and/or DMSO-d_6+D_2O at 400 MHz for ^{1}H and 100 MHz for ^{13}C; chemical shifts δ relative to int. standard TMS. 1D (^{1}H, ^{13}C) and 2D (COSY, HMQC, HMBC, ROESY) expts. were performed using standard Bruker DISNMR pulse program.

Isolation. (At almost every stage of fractioning, aliquots of the successive fractions were used for evaluation of the antiinflammmatory activity, to bio-guide the isolation work). Plant material (1 kg) was dried at room temperature and extracted with 95% EtOH. The solvent was removed under reduced pressure obtaining a residue (120 g). A part of the residue (59.4 g) was dissolved in a MeOH-H_2O (1:9/v/v) mixture. The solution was chromatographed on an Amberlite® column with MeOH-H_2O (1:9 and then 9:1) mixtures and finally with MeOH. Five fractions (I-V) were collected. Fraction III was re-chromatographed on a Sephadex LH-20 column using hexane-CH_2Cl_2-MeOH (2:1:0.5/v/v/v) as eluent. Fractions of 15 ml were collected and monitored by TLC. Repeated chromatography on an MPLC column with silica gel and gradient elution with hexane-EtOAc and EtOAc-MeOH, respectively, led to the isolation of **1** (12.5 mg).

Antineoplastic assays (San Feliciano *et al.*, 1993). A screening procedure was used to assess the antitumoral activity of comp **1** against the following neoplastic cell lines, P-388 (lymphoid neoplasm origin from DBA/2 mouse), A-549 (human lung carcinoma), HT-29 (human colon carcinoma), Mel-28 (malign melanom) and normal CV-1 (monkey kidney fibroblasts) cells.

Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of $1x10^4$ (P-388) and $2x10^4$ (A-549, HT-29) cells/well, respectively, in 1 ml aliquots of MEM 10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in absence of sample and counted daily to ensure the exponential growth of cells. After four days at 37°, under a 10% CO_2, 98% humid atmosphere, P-388 cells were observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls. A-549, HT-29, CV-1 and Mel-28 were stained with crystal violet before examination. As reference drug adriamycin was used. The global ethanol extract and the isolated compound **1** were evaluated in these cell lines.

The antioneoplastic studies were evaluated at Instituto BIOMAR S. A. (Cra de León, Carbajal, León, Spain).

Acknowledgments

To CYTED/SECAB for supporting this work and to Instituto BIOMAR S. A. for the cytotoxicity assays.

Cassels B., Urzúa A., Cortés M. and Garbarino J. (1973), Triterpenoids constituents of *Kageneckia oblonga*. Phytochemistry **12**, 3009–3010.

Delporte C., Backhouse N., Erazo S., Rojas J., Negrete R. and Muñoz O. (1998), Estudio comparativo de la toxicidad aguda y de los efectos analgésicos de dos especies de *Kageneckia* (Rosaceae). 4rd. Int. Symp. Natural Product Chemistry. Talca, Chile. (p. 197).

Delporte C., Backhouse N., Muñoz O., Erazo S., Negrete R., López-Pérez J. and San Feliciano A. (1997), Estudio Farmacológico de *Kageneckia oblonga*. Nuevo derivado de cucurbitacina de esta especie. Proceeding of the 15th. Congress Latinoam. Pharmac. and 6th Congress Interam. Clinic. Pharm. Therap. Cartagena de Indias, Colombia. Abstract Book p. 107.

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

Fikenscher N., Hegnauer Y. and Ruijgrok H. (1981), Die Verbreitung der Blausäure bei den Cormophyten: 15. Mitteilung. Neue Beobachtungen zur Cyanogenese bei den Rosaceen. Planta Med. **41**, 313–327.

Gallily R., Shohat B., Kalish J., Gitter S. and Lavie D. (1992), Further studies on the antitumor effect of cucurbitacins. Cancer Res. **22**, 1038–1045.

Gitter S., Gallily B., Shohat B. and Lavie D. (1961), Studies on the antitumor effect of cucurbitacins. Cancer Res. **21**, 516–521.

Hatam N. A., Whiting D. A. and Yousif N. J. (1989), Cucurbitacin glycosides from *Citrullus colocynthis*. Phytochemistry **28**, 1268–1271.

Maldonado S., Backhouse N., Delporte C., Negrete R., Erazo S., San Martín M. L., Ortíz de Urbina V., San Feliciano A. and García-Grávalos M. D. (1996a), Citotoxicidad y otras actividades biológicas de plantas chilenas antiinflamatorias y antipiréticas. *3rd. Int. Symp. Natural Product Chemistry.* Punta de Tralca, Chile. (p. 262).

Maldonado S., Delporte C., Backhouse N., Negrete R., Muñoz O., López-Pérez J. L. and San Feliciano A. (1996b), Efectos farmacológicos y compuestos mayoritarios de *Kageneckia oblonga*. 3rd. Int. Symp. Natural Product Chemistry. Punta de Tralca, Chile. (p. 264).

Muñoz M., Barrera E. and Meza I. (1981), El Uso Medicinal de Plantas Nativas y Naturalizadas en Chile. Museo Nacional Historia Natural. Publicación Ocasional. Santiago, Chile. p. 91.

Rodriguez G., Rodriguez R. and Barrales H. L. (1995), Plantas Ornamentales Chilenas. Editorial Aníbal Pinto. Concepción, Chile. p. 25.

San Feliciano A., Gordaliza M., Miguel del Corral J. M., Castro M., García-Grávalos M. D. and Ruiz-Lázaro P. (1993), Antineoplastic and antiviral activities of some cyclolignans. Planta Med. **59**, 246–249.

San Martín J. (1983), Medicinal Plants in Central Chile. Econ. Bot. **37**, 216–227.

Schenkel E. P., Farias M. R., Mayer R., Breitmaier E. and Rycker G. (1992), Cucurbitacins from *Wilbrandia ebracteata*. Phytochemistry **31**, 1329–1333.

Yesilada E., Tanaka S., Sezik E. and Tabata M. (1988), Isolation of antiinflamatory principle from the fruit juice of *Ecballium elaterium*. J. Nat. Prod. **51**, 504–508.