Socotroside, a New Pentacyclic Cucurbitane Glycoside from *Dendrosicyos socotrana*

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Phytochemical investigation of the ethyl acetate extract of the stem of *Dendrosicyos socotrana* Balf. f. resulted in the isolation of a new pentacyclic cucurbitane glycoside Socotroside, in addition to the three known cucurbitacins, dihydrocucurbitacin D, dihydrocucurbitacin F and cucurbitacin G. The structures of the isolated compounds were established on the basis of their spectral data. The isolated cucurbitacin aglycones showed marked cytotoxic activity.

Key words: *Dendrosicyos socotrana*, Cucurbitaceae, Pentacyclic Cucurbitane Glycoside, Cytotoxic Activity

Introduction

Dendrosicyos socotrana Balf. f. is a unique species known as cucumber tree native to Socotra island in the horn of Africa [1]. It belongs to Family Cucurbitaceae which is characterized by its contents of cucurbitacins [2, 3]. Cucurbitacins are a special group of highly oxygenated tetracyclic triterpenes having a double bond between C5 and C6 [4]. Several of these compounds possess a cyclized side chain, the mode of cyclization involving an ether linkage between C16-C24 [5, 6], C16-C23 [7, 8] or C20-C24 [9]. Biologically, they exhibit a wide range of bioactivity [10 – 12]. Recently, these compounds received a great deal of attention because of their cytotoxic and anticancer effects [13, 14]. A previous phytochemical study on the chloroform extract of the stem of the title plant has resulted in the isolation of dendrocyin [15] with a novel cyclic side chain. It was found of interest to continue this study on the ethyl acetate extract of this plant.

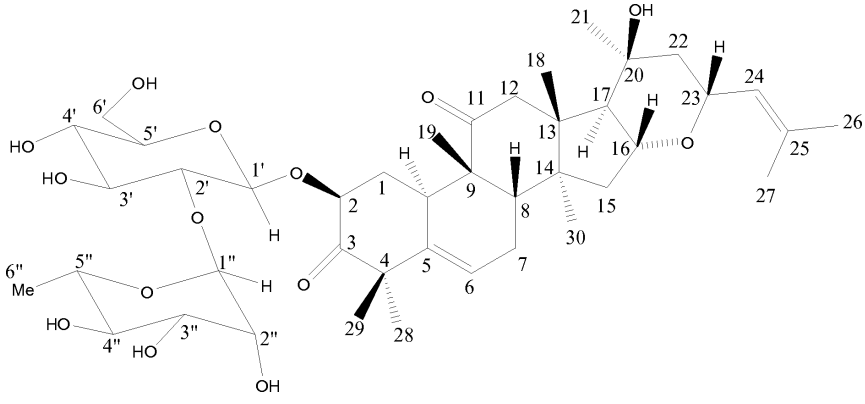
Discussion

Chromatographic fractionation of the ethyl acetate extract of *D. socotrana* Balf. f. stems on a silica gel column followed by reversed-phase silica gel column and HPLC separation yielded four cucurbitacins (**1** – **4**). Compounds **2** – **4** were identified as dihydrocucurbitacin D (**2**), 23, 24-dihydrocucurbitacin F (**3**) and cucurbitacin G (**4**) by comparison of their physical and spectroscopic properties with those reported in the literature [16 – 18].

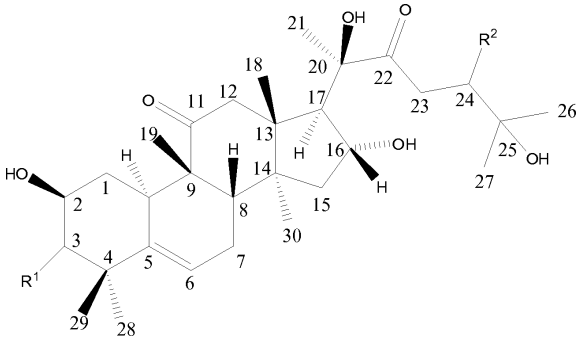
Compound **1** was isolated as a white amorphous powder with a positive optical rotation $[\alpha]_D^{25} = +32^\circ$, showing a violet spot on TLC when sprayed with 1 % $Ce(SO_4)_2$ in 10 % aq. H_2SO_4 followed by heating. The molecular formula $C_{42}H_{64}O_{14}$ was indicated from high-resolution positive FAB MS (*m/z* = 815.4281, calcd. 815.4279 for $C_{42}H_{64}O_{14}Na$, $[M+Na]^+$).

The ^{13}C NMR spectral data revealed the presence of two sugar moieties in addition to 30 signals for the aglycone moiety. Of the 11 degrees of unsaturation, implied by the molecular formula of compound **1**, two were accounted for carbon-carbon double bonds [δ = 137.9 (C5), 121.06 (C6), 142.0 (C25), 126.5 (C24)], two carbonyl groups [δ = 211.6 (C3) and 215.37 (C11)] which are characteristic for the cucurbitacin skeleton, as well as two sugar moieties. Because no other unsaturated function was indicated by the spectral data, the five remaining unsaturated functions were accounted to five rings, suggesting the presence of a pentacyclic triterpene derivative.

The ^1H NMR spectrum of **1** showed the presence of eight tertiary methyl groups (δ = 1.05, 1.14, 1.31, 1.27, 1.27, 0.96, 1.24, 1.29), two olefinic hydrogens [δ = 5.78 (H6, d, J = 5.3 Hz), 5.12 (H24, brs)], and two anomeric hydrogens $\big(\delta = 4.56$ (H-1′, d, J = 7.5 Hz) for a glucose moiety and 5.14 (H–1″, d, J= 1.1 Hz) for the rhamnose moiety$\big)$.



Socotroside (**1**)



R^1 = O, R^2 = H: Dihydrocucurbitacin D (**2**)
R^1 = ⋯OH, R^2 = H: 23,24-Dihydrocucurbitacin F (**3**)
R^1 = O, R^2 = OH: Cucurbitacin G (**4**)

The carbon signals of **1** in rings A, B and C closely match those of compound **4** (cucurbitacin G) and also those of other triterpenes [18], and the remaining carbon signals were in agreement with those reported for a 16α-,23-epoxy-cucurbitane-type triterpenes [7, 19].

The detailed analysis of COSY, HMQC and HMBC data confirmed the formulation of **1**. The relative stereostructure of **1** was characterized by careful comparison of the ^{13}C NMR data with those of rings A, B and C of compound **4**. The stereochemistry of the remaining rings D and E and the side chain was determined by different NOE experiments which showed NOE enhancement of β-oriented H18 and H23 on irradiation of H16 (δ = 4.15). The other important NOE correlations are shown in Fig. 1. The chemical shift values and coupling pattern of the protons at the positions C-16, C-17, C-22 and C-23 suggested a chair conformation for ring E.

Assignments, including all protons and carbons were confirmed by ^{1}H-^{1}H correlation spectroscopy (COSY and HMQC). The glycosylation shift was observed at C2 (Table 1). Furthermore, HMBC measurements (Fig. 2) helped in the unambiguous structural confirmation of the molecule including the site of attachment of the sugar at the cucurbitacin molecule, where the glucose unit was placed at C2 by the J_3 correlations between H1′ (δ = 4.56) and C2 (δ = 79.39), and the J_3 correlation between H1″ of the rhamnose (δ = 5.14) and C2′ of the glucose moiety.

The β-configuration at the anomeric center of the glucopyranose was suggested by the large coupling constant (J = 7.8 Hz) of the anomeric hydrogen at δ = 4.56 in the ^{1}H NMR spectrum, while, the α-configuration of rhamnose was assigned using the same tool (small coupling constant for the anomeric proton, J = 1.1 Hz).

From these lines of evidence, the structure of **1** was elucidated as 16α, 23α–epoxy–2, 20 β-dihydroxycucurbita– 5, 24-diene– 3, 11-dione 2-O [α-1 rhamnopyranosyl-(1→2)-α-D-glucopyranoside], which was named Socotroside.

The cytotoxic investigation of the isolated cucurbitacin aglycones (**2** – **4**) showed a significant activity against P_{388} leukemic cell lines with IC_{50} values of 1.5, 2.1 and 1.8 μg mL^{-1}, respectively. However, the cucurbitane glycoside (Socotroside **1**) has dis-

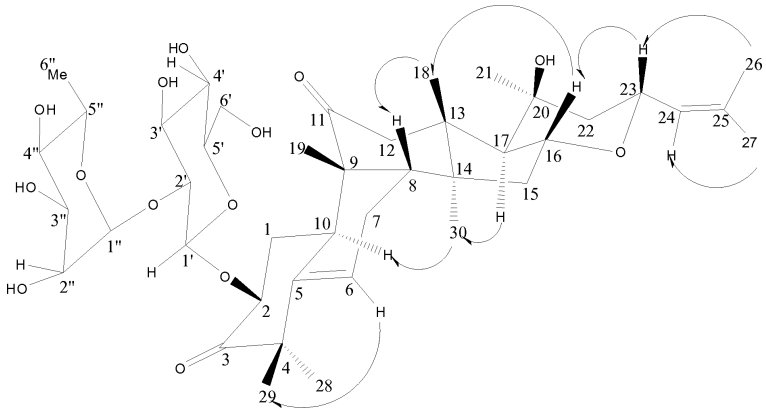


Fig. 1. Important NOE correlations of Socotroside (**1**).

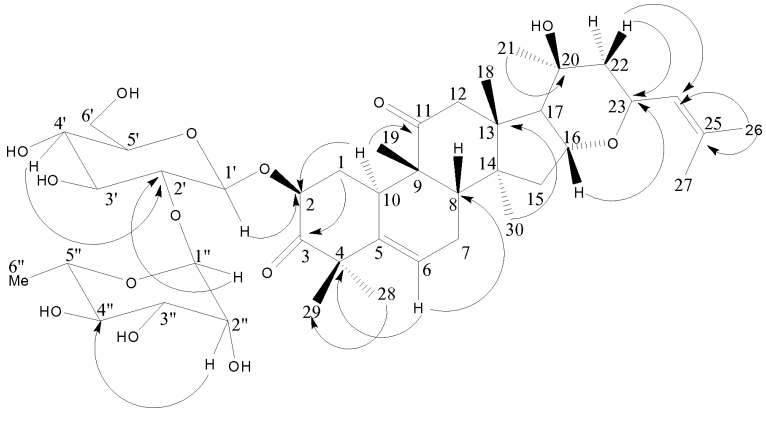


Fig. 2. Important HMPC correlations of Socotroside (**1**).

played a week cytotoxic activity against the same cell line.

Experimental Section

General experimental procedures

^{1}H and ^{13}C NMR (δ ppm, J in Hz): JOEL LNM 500 instrument (500/125 MHz) using CD_3OD as solvent (TMS as internal standard); EI MS: JOEL JMS GCMATE spectrometer; FAB MS: JOEL HX-110A mass spectrometer; IR: Shimadzu FTIR 8100 spectrometer.

Normal-phase column chromatography: silica gel BW 200 (Fujii Silysia chemical 230 – 400 mesh); Rp18 silica gel column chromatography: chromatorex ODS DM1020T (Fuji Silysia chemical, 100 – 200 mesh); HPLC: YMC pack ODS A (YMC, 250 × 20 mm id); HPLC detectors: Shimadzu RID 6A refractive index detector and Shimadzu OR-7 optical rotation detector. TLC: silica gel G F_{254} (Merck, 0.25 mm); solvent systems: $CHCl_3$-MeOH (95 : 5) and $CHCl_3$- MeOH-H_2O (10 : 3:1, lower layer); silica gel RP18 F_{254} (Merck, 0.25 mm), solvent systems: 35 %, 40 %, 55 % MeOH-H_2O and 25 % MeCN-H_2O. Spray reagent: 1 % $Ce(SO_4)_2$-10 %, aqueous H_2SO_4, followed by heating.

Plant material

The stems of *D. socotrana* Balf. f. were collected from Socotra island (March 2004) at the horn of Africa. A voucher specimen is deposited at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Egypt.

Extraction and isolation

The freshly sliced stems (1 kg) were extracted with boiling methanol (5 × 1.5 L) to complete exhaustion. The extract was concentrated in a rotary evaporator and fractionated by $CHCl_3$ and EtOAc. The EtOAc extract (35 g) was chromatographed on silica gel CC (900 g, *n*-hexane-EtOAc (10 : 1, 5 : 1, 1 : 1 and 1 : 10; v/v each 4 L), $CHCl_3$-MeOH-H_2O {10 : 3:1, 7 : 3 : 1 (lower layer) and 6 : 4 : 1; v/v/v each 4 L, then MeOH, 7.0 L}) to afford 8 fractions: fraction 1 (0 – 4.0 L, 6.8 g), fraction 2 (4.0 – 6.0 L, 0.56 g), fraction 3 (6.00 – 10.5 L, 6.8 g), fraction 4 (10.5 – 15.5 L, 4.1 g), fraction 5 (15.5 – 20.0 L, 5.2 g), fraction 6 (20.0 – 24.0 L, 0.11 g), fraction 7 (24.0 – 28.0 L, 1.8 g), and fraction 8 (28.0 – 35 L, 0.2 g).

Fraction 2 (0.56 g) was separated by reversed-phase silica gel column chromatography, 40 % MeOH-H_2O, and

Table 1. ^{1}H NMR (500 MHz, CD_3OD), ^{13}C NMR (125 MHz, CD_3OD), COSY, HMQC, DEPT, and HMBC data of compound **1**.

H	^{1}H NMR (δ in ppm)	J (Hz)	^{1}H-^{1}H COSY	C	^{13}C NMR (δ in ppm)	HMQC & DEPT	HMBC
1 α	1.39 dd	3.7, 14.0	H-1 β, H-2, H-10	**1**	35.6	C-1 α, C-1 β (CH_2)	C-3, C-5, C-10
1 β	1.39 dd	3.7, 14.0	H-1 α, H-2, H-10	**–**	–	–	–
2	3.44 m	–	H-1 α, H-1 β	**2**	79.4	CH-(O)	C-1-, C-1′
3	–	–	–	**3**	211.6	C=O	–
4	–	–	–	**4**	49.9	C	–
5	–	–	–	**5**	137.1	C(=)	–
6	5.78 d	5.3	H-7 α, H-7 β	**6**	121.1	CH-(=)	C-4, C-7, C-8, C-10
7- α	2.02 m	–	H-6, H-7 β, H-8	**7**	24.8	C-7 α, C-7 β (CH_2)	C-5, C-9, C-14
7- β	2.11 m	–	H-6, H-7 α, H-8	**–**	–	–	–
8	2.02 d	7.65	H-7 α, H-7 β	**8**	43.8	CH	C-11, C-13
9	–	–	–	**9**	49.3	C	–
10	2.95 m	–	H-1 α, H-1 β	**10**	35.1	CH	C-2, C-4, C-6
11	–	–	–	**11**	215.37	C=O	–
12 α	2.44 d	14.65	H-12 β	**12**	49.7	C-12 α, C-12 β (CH_2)	C-9, C-14
12 β	3.25 d	14.65	H-12 α	**–**	–	–	–
13	–	–	–	**13**	52.4	C	–
14	–	–	–	**14**	50.4	C	–
15 α	1.88 d	9.8	H-15 β, H-16	**15**	41.6	C-15 α, C-15 β (CH_2)	C-16, C-17
15 β	1.40 dd	3.7, 10.4	H-15 α, H-16	**–**	–	–	–
16	4.15 m	–	H-15 α, H-15 β, H-17	**16**	69.5	CH-(O)	C-15, C-20, C-23
17	1.98 bd	9.3	H-16	**17**	56.56	CH	C-21, C-22
18	1.05 s	–	–	**18**	20.33	CH_3	C-13, C-17
19	0.96 s	–	–	**19**	20.22	CH_3	C-9
20	–	–	–	**20**	72.1	C-(O)	–
21	1.72 s	–	–	**21**	25.9	CH_3	C-17, C-20
22 α	1.45 m	–	H-22 β, H-23	**22**	41.63	C-22 α, C-22 β (CH_2)	C-17, C-24
22 β	1.53 m	–	H-22 α, H-23	**22**	49.6	–	–
23	4.96 dd	7.2, 12.9	H-22 α, H-22 β, H-24	**23**	76.3	C-(O)	C-20, C-25
24	5.12 brs	–	H-23	**24**	126.5	CH-(=)	C-26, C-27
25	–	–	–	**25**	142.0	C (=)	–
26	1.31 s	–	–	**26**	21.4	CH_3	25
27	1.27 s	–	–	**27**	29.3	CH_3	25
28	1.29 s	–	–	**28**	29.1	CH_3	C-3, C-4, C-5
29	1.24 s	–	–	**29**	22.0	CH_3	C-3, C-4, C-5
30	1.14 s	–	–	**30**	17.9	CH_3	C-8, C-15
Glu							
1′	4.56 d	7.5	H-2′	**1′**	100.8	CH-(O)	C-2′
2′	4.41 m		H-1′, H-3′	**2′**	79.2	CH-(O)	C-1′, C-1″
3′	3.18 m	–	H-2′, H-4′	**3′**	77.64	CH-(O)	C-1′, C-5′
4′	3.34 m	–	H-3′, H-5′	**4′**	74.2	CH-(O)	C-2′, C-6′
5′	3.39 m		H-4′, H-6 α, H-6 β	**5′**	78.0	CH-(O)	C-1′, C-3′
6′ α	3.59 q	6.1, 11.6	H-5′, H-6 β	**6′**	63.1	C-6′ α, C-6′ β (CH_2)	C-4′, C-5′
6′ β	3.85 dd	1.5, 11.6	H-5′, H-6 α				–
Rham							
1″	5.14 d	1.1	H-2″	**1″**	102.4	CH-(O)	C-2′, C-3″
2″	3.95 m		H-1″, H-3″	**2″**	72.4	CH-(O)	C-1″, C-2″
3″	3.82 m		H-2″, H-4″	**3″**	72.3	CH-(O)	C-1″, C-5″
4″	3.32 m		H-3″, H-5″	**4″**	74.5	CH-(O)	C-1″
5″	3.14 m		H-4″, H-6″	**5″**	72.3	CH-(O)	C-1″, C-4″, C-5″
6″	1.71 d	6.1	H-5″	**6″**	18.5	CH_3	C-5″, C-4″

HPLC (Rp18, 48 % MeOH-H_2O) to afford compound **2** (40 mg).

Fraction 4 (4.1 mg) was separated by RpC-18 silica gel column chromatography (42 % MeOH-H_2O) and HPLC (ODS, Rp18, 50 % MeOH-H_2O) to afford compound **3** (80 mg).

Fraction 6 (110 mg) was separated by RpC-18 silica gel column chromatography (45 % MeOH-H_2O) and HPLC

(ODS, Rp18, 32 % MeCN-H_2O) to afford compound **4** (35 mg).

Fraction 7 (1.8 g) was separated by reversed-phase silica gel column chromatography (65 % MeOH-H_2O) and HPLC (ODS, RpC-30, 80 % MeCN-H_2O) to afford compound **1** (62 mg).

Identity of the sugar moiety of socotroside

Socotroside (2 mg) was subjected to acid hydrolysis by refluxing with 1 M HCl (1 mL) at 80 °C for 3 h followed by neutralization with Ag_2O. The hydrolysate was separated into the aqueous layer after washing with EtOAc and tested for the identity of the sugar moiety. The identity of liberated sugars as D-glucose and L-rhamnose was confirmed by TLC using *n*-BuOH-pyridine-HOAc-EtOAc-H_2O (5 : 2 : 1 : 2.5, double run) as a solvent system and HPLC using 85 % CH_3CN-H_2O as a solvent system (Kasei sorb LC NH_2 60 S). The detection was achieved using an optical rotation detector. The t_R value for the detected sugars were 9.1 (–) and 18.6 (+) min confirming the presence of rhamnose and glucose, respectively.

Cytotoxic activity

Cytotoxic activity was measured on P_{388} using the standard procedure as described in ref. [20].

Identification of isolated compounds

*Socotroside (**1**)*

White amorphous powder. – Prep. HPLC, t_R = 31 min. – $[\alpha]_D^{24}$ = +32 °C (c = 0.25°, MeOH). – IR (KBr): ν = 3540 (OH), 1690 (C=O), 1640 (C=C), 1265, 1070 cm^{-1}. – HRMS ((+)–FAB): m/z = 815.4281 (calcd. 815.4279 for $C_{42}H_{64}O_{14}Na$, $[M+Na]^+$), FAB MS: m/z = 793 $[M+1]^+$. – ^1H, ^{13}C NMR, DEPT, COSY, HMQC and HMBC data are listed in Table 1.

*Dihydrocucurbitacin D (**2**)*

White needle-shaped crystals from EtOAc, m. p. 167 °C. – Prep. HPLC: t_R = 22 min, $[\alpha]_D^{24}$ = +82° (c = 0.25, MeOH). – IR (KBr): ν = 3400 (OH), 1692 (C=O), 1642 (C=C) cm^{-1}. – FAB MS: m/z = 519 $[M+1]^+$. – ^1H NMR (500 MHz, $CDCl_3$, 24 °C, TMS): δ = 5.74 (br.s, 1H, 6-H), 4.39 (dd, ^2J = 12.0 Hz, ^3J = 6.0 Hz, 1H), 3.22 (d, J = 14.8 Hz, 1H, 12, a-H), 2.68 (d, J = 14 Hz, 1H, 8, 12b-H), 4.30 (d, J = 7.1 Hz, 1H, 1, 6-H), 0.97 (s, 3H, Me-18), 1.07 (s, 3H, Me-19), 1.37 (s, 3H, Me-21), 1.22 (CH_3, 3H, Me-26), 1.25 (CH_3, 3H, Me-27), 1.39 (CH_3, 3H, Me-28), 1.28 (CH_3, 3H, Me-29), 1.08 (CH_3, 3H, Me-30). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 36.0 (CH_2, C1), 71.6 (CH, C2), 212.0 (C, C3), 50.3 (C, C4), 140.2 (C, C5), 120.3 (CH, C6), 23.9 (CH_2, C7), 42.4 (CH, C8), 48.4 (C, C9), 33.7 (CH, C10), 212.8 (C, C11), 48.7 (CH_2, C12), 48.3 (C, C13), 50.7 (C, C14), 45.4 (CH_2, C15), 70.9 (CH, C16), 57.7 (CH, C17), 19.9 (CH_3, C18), 18.9 (CH_3, C19), 79.2 (C, C20), 24.6 (CH_3, C21), 215.2 (C, C22), 30.9 (CH_2, C23), 36.9 (CH_2, C24), 70.3 (C, C25), 28.7 (CH_3, C26), 29.9 (CH_3, C27), 21.3 (CH_3, C28), 29.3 (CH_3, C29) and 20.1 (CH_3, C30). These data are in agreement with those reported in the literature for dihydrocucurbitacin D (**2**) [3].

*23,24-Dihydrocucurbitacin F (**3**)*

White needles, m. p. 145 – 147 °C. – Prep. HPLC: t_R = 17 min, $[\alpha]_D^{24}$ = +56° (c = 0.25, MeOH). – IR (KBr): ν = 3650 (OH), 1695 (C=O), 1643 (C=C) cm^{-1}. – FAB MS: m/z = 521 $[M+1]^+$. – ^1H NMR (500 MHz, $CDCl_3$, 24 °C: TMS): δ = 4.4 (t, J = 7.7 Hz, 1H, 2H), 3.9 (d.-like, J = 8.2 Hz 1H), 5.65 (br. d, J = 5.4 Hz, 1H,), 4.95 (m, 1H, 16-H), 0.89 (s, 3H, Me-18), 1.26 (s, 3H, Me-19), 1.37 (s, 3H, Me-21), 1.28 (s, 3H, Me-26), 1.29 (s, 3H, Me-27), 1.18 (s, 3H, Me-28), 1.11 (s, 3H, Me-29), 1.04 (s, 3H, Me-30). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 33.06 (CH_2, C1), 69.2 (CH, C2), 79.9 (CH, C3), 42.7 (C, C4), 139.8 (C, C5), 121.0 (CH, C6), 24.8 (CH_2, C7), 44.4 (CH, C8), 49.2 (C, C9), 34.94 (CH, C10), 216.3 (C, C11), 49.9 (CH_2, C12), 51.8 (C, C13), 50.2 (C, C14), 46.7 (CH_2, C15), 71.4 (CH, C16), 59.2 (CH, C17), 20.4 (CH_3, C18), 19.6 (CH_3, C19), 80.8 (C, C20), 27.2 (CH_3, C21), 217.2 (C, C22), 38.08 (CH_2, C23), 29.9 (CH_2, C24), 70.8 (C, C25), 29.4 (CH_3, C26), 29.2 (CH_3, C27), 20.4 (CH_3, C28), 25.5 (CH_3, C29), 26.2 (CH_3, C30). These data are in agreement with those reported in the literature for 23,24-Dihydrocucurbitacin F (**3**) [16].

*Cucurbitacin G (**4**)*

White powder from MeOH. – Prep. HPLC: t_R = 21 min, $[\alpha]_D^{24}$ = +70.9° (c = 0.076, $CHCl_3$). – IR (KBr): ν = 3480 (OH), 1692 (C=O), 1650 (C=C) cm^{-1}. – FAB.MS: m/z = 535 $[M+1]^+$, 557 $[M+Na]^+$. – ^1H NMR (500 MHz, $CDCl_3$, 24 °C, TMS): δ = 4.35 (dd, ^2J = 12.5 Hz, ^3J = 6.2 Hz, 1H, 2-H), 5.72 (br. d, J = 5.8 Hz, 1H, 6-H), 3.22 (d, J = 14.8 Hz, 1H, 12a-H), 2.62 (d, J = 14.8 Hz, 1H, 12b-H), 4.28 (br. s, 1H, 16-H), 2.89 (dd, ^2J = 9.8 Hz, ^3J = 16.5 Hz, 1H, 23a-H), 2.64 (dd, ^2J = 1.6 Hz, ^3J = 16.5 Hz, 1H, 23b-H), 3.9 (br. d, J = 9.8 Hz, 1H, H-24), 0.91 (s, 3H, Me-18), 1.1 (s, 3H, Me-19), 1.32 (s, 3H, Me-21), 1.13 (s, 3H, Me-26), 1.6 (s, 3H, Me-27), 1.28 (s, 3H, Me-28), 1.22 (s, 3H, Me-29), 1.06 (s, 3H, Me-30). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 36 (CH_2, C1), 71.3 (CH, C2), 212.2 (C, C3), 50.3 (C, C4), 140.5 (C, C5), 120.3 (CH, C6), 23.8 (CH_2, C7), 42.2 (CH, C8), 48.4 (C, C9), 33.7 (CH, C10), 213.0 (C, C11), 48.6 (CH_2, C12), 50.6 (C, C13), 48.3 (C, C14), 45.4 (CH_2, C15), 71.0 (CH, C16), 57.4 (CH, C17), 19.8 (CH_3, C18), 20.0 (q, C19), 79.4 (s, C20), 24.2 (q, C21), 215.5 (s, C22), 38.3 (t, C23), 74.3 (d, C24), 72.3 (C, C25), 24.6 (CH_3, C26), 25.7 (CH_3, C27), 21.3 (CH_3, C28), 29.2 (CH_3, C29) and 18.4 (CH_3, C30). These data are in agreement with those reported in the literature for Cucurbitacin G (**4**) [17].

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