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Dendrocyin: an isocucurbitacin with novel cyclic side chain from *Dendrosicyos socotrana*

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Abstract

Dendrosicyos socotrana Balf.f. is a unique species (Cucurbitaceae) native to Socotra island in the horn of Africa. From the chloroform extract of the stems, A new isocucurbitacin (Dendrocyin) with unusual cyclization in the side chain; 24β-ethoxy-20-25-epoxy-3α,16α-dihydroxy-9-methyl-19-norlanost-5(6) ene-2,11,22-trione has been isolated alongside isocucurbitacin R. Their structural configuration were established by usual spectroscopic (^{1}H NMR, ^{13}C NMR and DEPT) and two-dimensional NMR techniques (^{1}H–^{1}H Cosy, HMBC and HMQC).

Keywords: *Dendrosicyos socotrana* Balf.f.; Cucurbitaceae; Stems; Isocucurbitacins

1. Introduction

Cucurbitacins are special group of highly oxygenated tetracyclic triterpenes with a wide range of bioactivity (Lavie and Glotter, 1971; Doskotch et al., 1969; Smit et al., 2000; Cassady and Suffness, 1980; Ito et al., 2002 and Dinan et al., 1997). Several cucurbitacins belonging to different families were previously isolated possessing a cyclized side chain. The mode of cyclization was found through an ether linkage between C16–C24 (Hylands and Mansour, 1983 and Gamlath et al., 1988), C16–C23 (Schenkel et al., 1992; Kanchanapoom et al., 2002) and C20–C24 (Stuppner and Muller, 1993).

In continuation of our investigation for isolation of new bioactive compounds from natural sources, the present study deals with the isolation and structure elucidation of a new member of isocucurbitacins showing unusual mode of cyclization in the side chain from *Dendrosicyos socotrana* Balf.f. *D. socotrana* Balf.f is a unique species known as cucumber tree, belongs to the family Cucurbitaceae and endemic in Socotra island. It is a tree has a swollen trunk with a whitish gray bark and pendant fleshy branches. The leaf is pubescent has prickly margin without tendrils and possessing a distinctly bitter taste (Miller and Bazara'a, 1996).

2. Results and discussion

Chromatographic fractionation of the chloroformic extract of *D. socotrana* Balf.f stems on silica gel opened column followed by reversed-phase silica gel column

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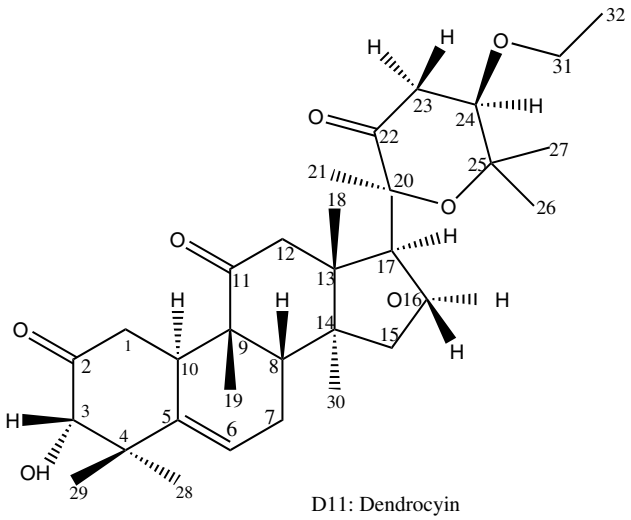
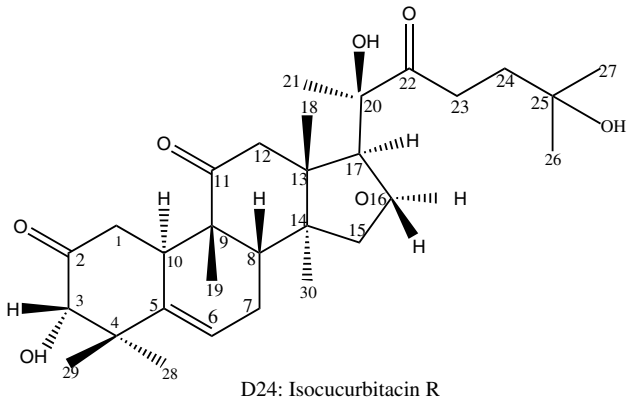
and HPLC separation yielded two cucurbitacins give brown colour with vanillin/H_2SO_4 spray reagent (D11 and D24).



D11: Dendrocyin



D24: Isocucurbitacin R

Compound D24 was obtained as a white prisms with the molecular formula $C_{30}H_{46}O_7$ (EI-MS; *m/z* 518 M^+). The MS also shows the fragment at *m/z* 500 indicating a subsequent loss of H_2O and a base peak at *m/z* 113 characteristic to a cucurbitacin with saturated side chain (Duncan et al., 1968). ^{1}H NMR showed a sharp singlet at δ3.90 assigned to α-hydroxylated methine group indicating the presence of a compound of the isocucurbitacin series with 2-keto-3-α hydroxy system (Kupchan

Table 1
Spectral data of isocucurbitacin R (D24) ^{1}H NMR(500 MHz; CDOD) and ^{13}C NMR(125 MHz; CDOD)

H	^{1} H NMR δ ppm	*J* (Hz)	C	^{13} C NMR δ ppm	HMQC & DEPT	H–H-Cosy	HMBC[a]
1α	2.27 (*m*)	–	1	39.77	C-1α, C-1β(CH_2)	H-1β, H-3, H-10	C-2, C-3, C-5, C-10
1β	2.79 (*m*)	–	2	211.13	C=O	H-1α, H-3, H-10	–
3	3.94 (*s*)	–	3	80.80	CH–(O)	H-1α	C-1, C-2, C-5, C-28, C-29
6	5.98 (*d*)	5.90	4	47.07	C	H-7α, H-7β	–
7α	2.06 (*d*)	7.56	5	138.95	C(=)	H-6, H-7β, H-8	–
7β	2.48 (*d*)	7.56	6	122.17	CH-(=)	H-6, H-7α, H-8	C-4, C-8, C-10
8	2.10 (*d*)	7.56	7	24.34	C-7α, C-7β(CH_2)	H-7α,β	C-6, C-8
10	2.80 (*m*)	–	8	43.66	CH	H-1α, H-1β	C-6, C-7, C-10, C-13, C-14, C-30
12α	3.26 (*d*)	14.85	9	48.50	C	H-12β	–
12β	2.59 (*d*)	14.85	10	36.88	CH	H-12α	C-1, C-11
15α	1.85 (*dd*)	12.70, 9.20	11	214.30	C=O	H-16, H-15β	–
15β	1.40 (*m*)	–	12	49.79	C-12α, C-12β(CH_2)	H-15α	C-11, C-13, C-14, C-18
16	4.35 (*t*)	7.83	13	51.11	C	H-15α, H-17	–
17	2.55 (*d*)	7.02	14	49.20	C	H-16	–
23α	2.83 (*dd*)	6.20, 9.50	15	45.99	C-15α, C-15β(CH_2)	H-24α	C-16
23β	2.69 (*dd*)	6.20, 9.50	16	70.74	CH–(O)	H-24α	C-17, C-20, C-14, C-18
24α	1.70 (*t*)	7.56	17	58.50	CH	H-23α,β	C-16, C-22.
24β	n	–	18	20.20	CH_3	–	C-8, C-11, C-12, C-14
Me-18	1.16 (*s*)	–	19	19.23	CH_3	–	C-8, C-11
Me-19	1.28 (*s*)	–	20	80.83	C–(O)	–	–
Me-21	1.43 (*s*)	–	21	25.05	CH_3	–	C-17, C-20
Me-26	1.19 (*s*)	–	22	216.10	C=O	–	–
Me-27	1.19 (*s*)	–	23	32.34	C-23α, C-23β(CH_2)	–	C-22, C-24
Me-28	0.84 (*s*)	–	24	37.54	C24α, C24β(CH_2)	–	C-23, C-25, C-26, C-27
Me-29	1.31 (*s*)	–	25	70.27	C–(O)	–	–
Me-30	0.93 (*s*)	–	26	29.12	CH3	–	C-24, C-25, C-27
			27	29.04	CH_3		C-24, C-25, C-26
		28	21.41	CH_3		C-3, C-4, C-5	
			29	24.34	CH_3		C-3, C-4, C-5
			30	20.27	CH_3		C-13, C-14

n, overlapped signal.
[a] HMBC correlations of some protons could not be traced.

Table 2
Spectral data of dendrocyin (D11) ^{1}H NMR (500 MHz; CDOD) and ^{13}C NMR (125 MHz, CDOD)

H	^{1} H NMR δ ppm	J (Hz)	C	^{13}C NMR δ ppm	HMQC & DEPT	^{1}H–^{1}H-COSY	HMBC[a]
1α	2.24 (*dd*)	5.90, 15.70	1	39.92	C-1α, C-1β(CH_2)	H-1β, H-10	C-2, C-3, C-5, C-10
1β	2.80 (*m*)	–	–	–	–	H-1α	–
–	–	–	2	211.26	C=O	–	–
3	3.94 (*s*)	–	3	80.99	CH–(O)	–	C-1, C-2, C-4, C-28, C-29
–	–	–	4	47.18	C	–	–
–	–	–	5	139.22	C(=)	–	–
6	5.96 (*d*)	6.00	6	122.26	CH(=)	H-7α, H-7β	C-4, C-7, C-8
7α	2.03 (*d*)	7.56	7	24.47	C-7α, C-7β(CH_2)	H-7β,6	C-6
7β	2.51 (*d*)	7.29	–	–	–	H-7α,6	–
8	2.09 (*d*)	7.56	8	43.87	CH	H-7α,β	C-10
–	–	–	9	48.70	C	–	–
10	2.80 (*t*)	8.64	10	37.04	CH	H-1α, H-1β	C-2
–	–	–	11	214.37	C=O	–	–
12α	3.23 (*d*)	14.85	12	49.49	C-12α, C-12β(CH_2)	H-12β	C-9, C-11, C-13, C-18
12β	2.57 (*d*)	14.85	–	–	–	H-12α	–
–	–	–	13	51.40	C	–	–
–	–	–	14	49.37	C	–	–
15α	1.84(*dd*)	9.18, 12.96	15	46.17	C-15α, C-15β(CH_2)	H-15β, H-16	C-13, C-17
15β	1.43 (*dd*)	9.18, 12.96	–	–	–	H-15α, H-16	–
16	4.36 (*t*)	7.56	16	70.97	CH–(O)	H-15, H-17	C-13, C-14, C-17, C-20
17	2.50 (*d*)	7.29	17	58.23	CH	H-16	C-15, C-18, C-20
–	–	–	18	20.23	CH_3	–	C-13, C-14, C-17
–	–	–	19	19.30	CH_3	–	–
–	–	–	20	80.22	C–(O)	–	–
–	–	–	21	24.84	CH_3	–	C-22
–	–	–	22	214.50	C=O	–	–
23α	3.0 (*dd*)	2.70, 17.60	23	39.83	C23α, C-23β(CH_2)	H-23β, H-24	C-22, C-24
23β	2.76 (*dd*)	8.50, 17.60	–	–	–	H-23, H-24	–
24α	3.68 (*dd*)	2.70, 8.50	24	83.15	CH–(O)	H-23α, H-23β	C-25, C-31
Me-18	1.16 (*s*)	–	25	73.44	C–(O)	–	–
Me-19	1.31 (*s*)	–	26	25.08	CH_3	–	C-24
Me-21	1.39 (*s*)	–	27	26.48	CH_3	–	C-24, C-25, C-26
Me-26	1.16 (*s*)	–	28	21.49	CH_3	–	C-3, C-4, C-29
Me-27	1.19 (*s*)	–	29	24.40	CH_3	–	C-3, C-4, C-28
Me-28	0.84 (*s*)	–	30	20.46	CH_3	–	–
Me-29	1.28 (*s*)	–	31	68.70	CH_2-(O)	–	C-24
Me-30	0.93 (*s*)	–	32	15.88	CH_3	–	C-31
Me-32	1.08 (*t*)	6.75	–	–	–	–	–
CH_2-31	3.60 (Q)	6.75, 13.77	–	–	–	–	–

[a] HMBC correlations of some protons could not be traced.

et al., 1978; Cattel et al., 1978 and Hyands and Maged, 1986). The assignments of the different protons at C-1, C-7, C-8, C-10, C-12, C-15, C-16, C-17, C-23 and C-24 and their coupling constants were established by means of a ^{1}H–^{1}H Cosy experiment.

^{13}C NMR and DEPT experiments (Table 1) showed 30 carbon signals attributed to three carbonyls, seven quaternary carbons, six methines, six methylenes and eight methyls. The rest of the molecule and ^{1}H–^{13}C connectivities was further established by HMQC and HMBC experiments. As a result compound D24 was identified as isocucurbitacin R (tetrahydro-isocucurbitacin I, dihydro-isocucurbitacin D) a rare isocucurbitacin previously synthesized from cucurbitacin I and was believed to be an artifact from dihydro-cucurbitacin D (Cattel et al., 1978).

Compound D11 was obtained as white prisms with the molecular formula $C_{32}H_{48}O_7$ (MS and ^{13}C NMR). EI-MS showed a fragment at *m*/*z* 498 (1.3) corresponding to $M^+ - 46$ indicating subsequent loss of ethanol followed by the common cucurbitacin fragments at *m*/*z* 403 (1.4), 385 (2.7) and a base peak at *m*/*z* 96 (C_6H_8O) indicating the loss of a cucurbitacin side chain (Audier and Das, 1966).

The IR indicated the presence of a hydroxyl, carbonyls and an olifenic functionalities. ^{1}H NMR (Table 2) showed a singlet at δ 3.94 (*s*, H-3β) confirmed the presence of a cucurbitacin with 2-keto-3α-hydroxy system in ring A indicating a member of iso-cucurbitacin series (Kupchan et al., 1978; Cattel et al., 1978 and Hyands and Maged, 1986). Furthermore, It showed an olifinic proton at δ 5.96 (*d*, $J = 6.0$ Hz, H-6), an

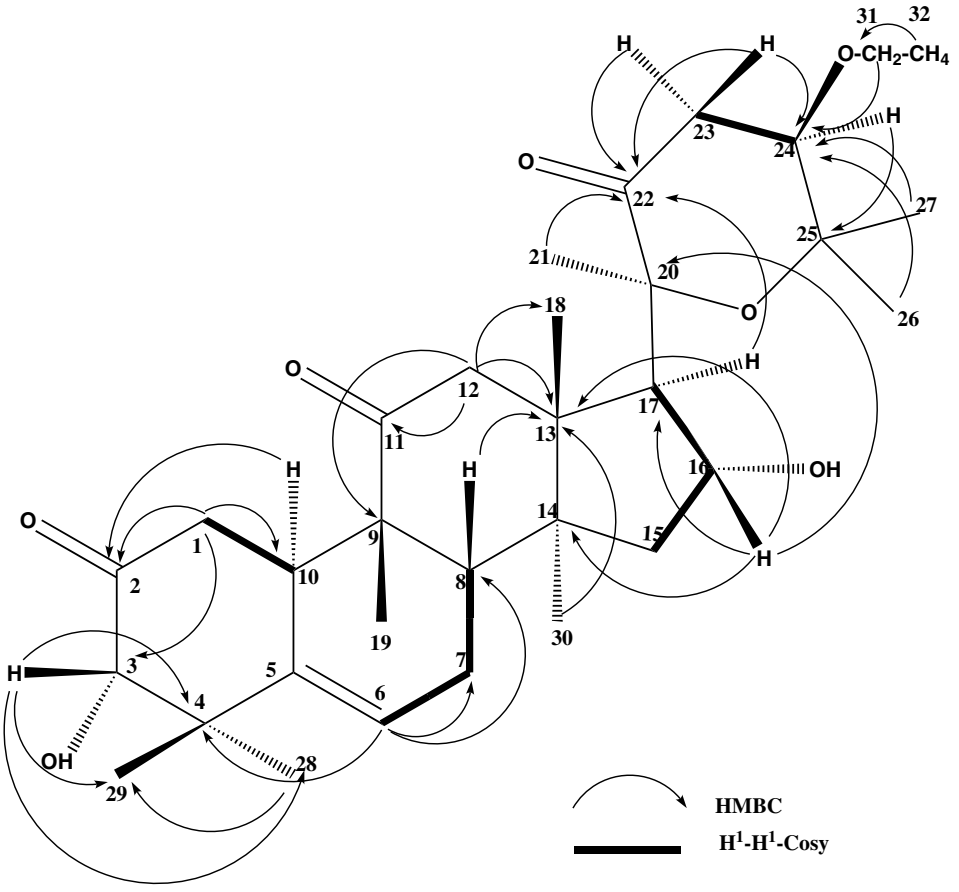


Fig. 1. HMBC and ^{1}H–^{1}H Cosy for D11 (dendrocyin).

α-hydroxyl group at C-16 and an AB system at δ 2.59 (H-12β, *d*, *J* = 14.85 Hz) and 3.23 (H-12α, *d*, *J* = 14.85 Hz) super imposable on those of isocucurbitacin R (Table 1). In addition, It showed nine methyl signals in the range δ 0.84–1.39, all of them are singlets except a triplet of methyl protons at δ 1.08 (*t*, *J* = 6.75 Hz) coupled with a downfield methylene protons at δ 3.60 (*q*, *J* = 6.75, 13.77 Hz) indicating the presence of an ethoxy group. The assignments of the protons at C-1, C-7, C-8, C-10, C-15 and C-17 and their coupling constants were determined by means of ^{1}H–^{1}H Cosy experiment (Table 2). ^{13}C NMR and DEPT experiments indicated the presence of 9 methyls, 6 methylenes, 7 methines and 10 quaternary carbons. The carbon signals due to A–D rings were almost super imposable on those of isocucurbitacin R. However, the carbon signals associated with the side chain showed differences in their multiplicities as well as their chemical shift values. It showed two oxygenated quaternary carbons at δ 80.22 and 73.44, one oxygenated methine (δ 83.15), one methylene (δ 39.83), one carbonyl carbon (δ 214.50), three methyl singlets (δ 24.84, 25.08 and 26.48) in addition to an ethoxy group (δ 15.88 and 68.70) (Table 2).

The molecular formula and the ratio of carbon to hydrogen indicated 9 unsaturation centers and suggested the presence of a side chain cyclized via an ether linkage. HMQC and ^{1}H–^{1}H Cosy experiments allowed the assignment of all carbons directly bounded to the protons in the cyclized side chain (Table 2).

The structure of the cyclized side chain was deduced from HMBC experiment (Fig. 1 and Table 2). The oxygenated methine carbon at δ 83.15(C-24) showed three bond coupling (^{3}J) with the two methyl protons at δ 1.19 and 1.16 (C-26 and C-27 methyls), methylene protons of the ethoxy group at δ 3.6 and two bond coupling (^{2}J) with the methylene protons at δ 2.76 and 3.03 (C-23). The carbonyl carbon at δ 214.5 (C-22) displayed two bond connectivity (^{2}J) with the methylene protons at δ 2.76 and 3.03, three bond coupling (^{3}J) with the methyl protons at δ 1.39 (C-21) and methine proton at δ 2.5 (C-17). Taken together these data supported the location of the ether bridge between the two quaternary carbons C-20 and C-25 and allowed the description of the planer structure of D11. This was further substantiated by the presence of a base peak at *m*/*z* 96 (characteristic for cucurbitacins with C23–C24 double bond, Audier and Das, 1966)

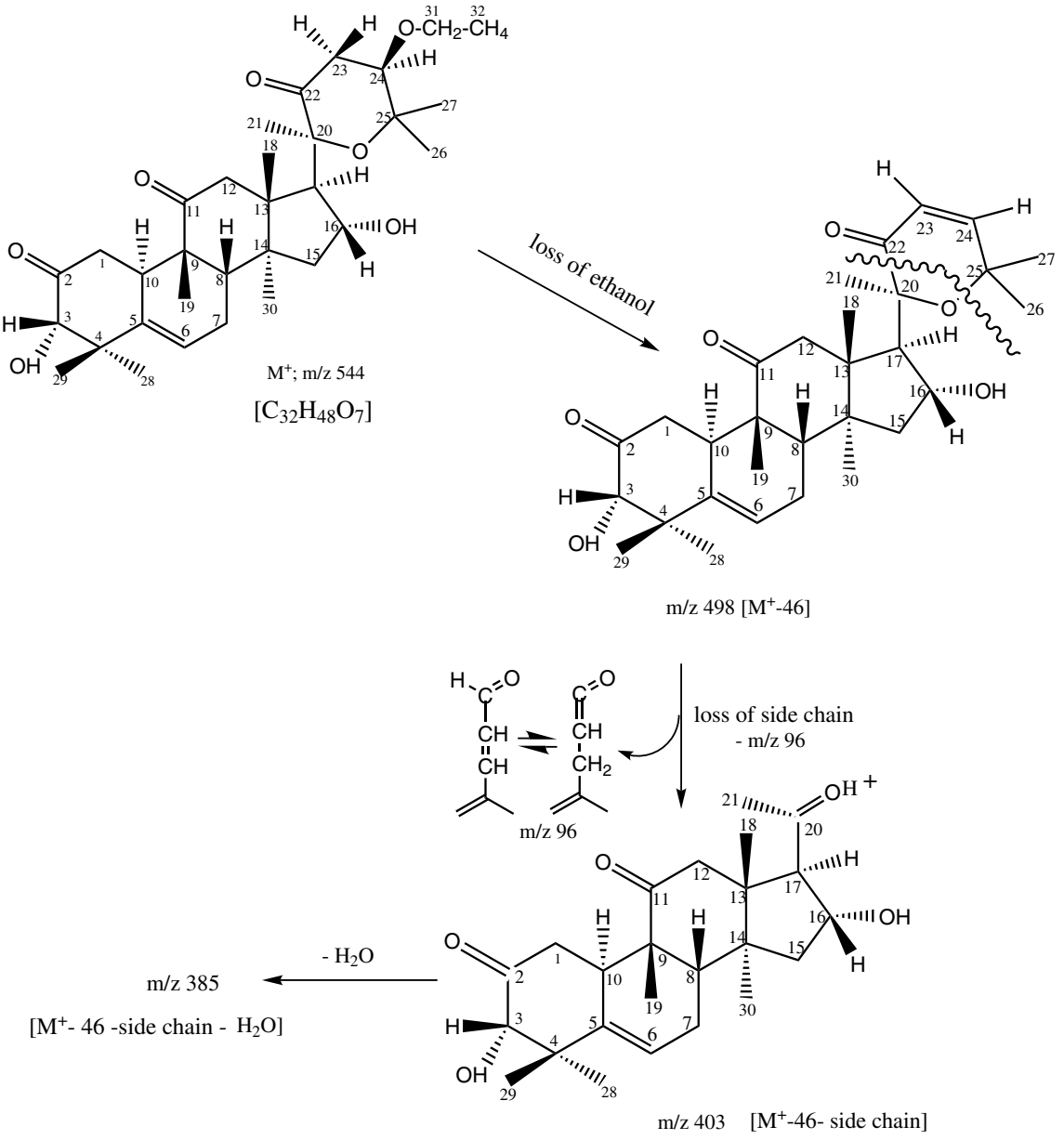


Fig. 2. Mass fragmentation pattern of D11 (dendrocyin).

in the MS which explained the formation of the double bond between C-23 and C-24 after the loss of the ethoxy group at C-24 and followed by cleavage of the side chain ($M^+ - 46–96$) (Fig. 2) (Audier and Das, 1966).

The stereochemistry of the methine proton at C-24 was deduced from the coupling constant between C-23 and C-24 protons (Table 2) to be H-24α(ax) and 24β(eq)-ethoxy configuration. Thus dendrocyin is 24β-ethoxy-20-25 epoxy-3α,16α-dihydroxy-9-methyl-19-norlanost-5(6) ene-2,11,22-trione.

Biogenetically, dendrocyin could originate from isocucurbitacin G or 24-oxygenated derivatives of iso-cucurbitacin R by spontaneous dehydration/cyclization of C20 and C25 hydroxyl groups.

3. Experimental

3.1. General

^1H and ^{13}C NMR (δ ppm, J in Hz), JOEL–LNM-500 with a 500/125 MHz using CD_3OD (TMS as int. standard); EI-MS and HREI-MS, JEOL JMS-GCMATE spectrometer; IR, Shimadzu FTIR-8100; Normal phase CC used silica gel BW-200 (Fuji Silysia Chemical,

230–400 mesh); Reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, 100–200 mesh), HPLC, YMC-Pack ODS-A (YMC, 250 × 20 mm i.d.); TLC: silica gel $60F_{254}$, (Merck, 0.25 mm), Solvent systems: $CHCl_3$–MeOH (95:5), Spray reagent: vanillin/H_2SO_4 followed by heating.

3.2. Plant material

The stems of *D. socotrana* Balf.f were collected from Socotra island (March, 2002) in the horn of Africa. A voucher specimen is deposited at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Egypt.

3.3. Extraction and isolation

The fresh sliced stem (1 kg) was exhausted by boiling methanol (3 × 1.5 L) and the concentrated extract was fractionated by $CHCl_3$ and EtOAC. The $CHCl_3$ extract (25.5 g) was chromatographed on opened column of silica gel (500 g) using $CHCl_3$–MeOH gradient mixture as eluent. 75 Fractions of 100 ml each were collected and monitored by TLC.

Fractions 23–34 (1.2 g) eluted by 4.5% MeOH/$CHCl_3$ indicated the presence of two major cucurbitacins (brown colour with vanillin/H_2SO_4) was further purified by reversed-phase silica gel column (32 g, MeOH–H_2O 57:43 v/v, MeOH) and HPLC (MeOH–H_2O 62:28 v/v; flow rate, 1 ml/min; detection, refractive index) to afford D11 (68 mg) and D24 (133 mg).

3.4. Identification of the isolated compounds

3.4.1. Compound D24 (isocucurbitacin R)

White prisms (from methanol), m.p. 187–190°, tlc: R_f 0.55, HPLC: t_R 43.1 min. EI-MS *m/z* (rel.int.): 518 (0.3) [M^+], 500(1.7) [$M^+ - H_2O$], 482 (10.4), 403 (34.8) [M^+ – side chain], 385 (29.5) [M^+ – side chain H_2O], 369 (14.5), 367 (10.5), 237 (8.5), 219 (12.1), 166 (28), 113 (100), 87 (16.5), 69 (18.1) and 55 (16.2).

^{1}H NMR, ^{13}C NMR, HMQC and HMBC data: see Table 1.

3.4.2. Compound D11 (dendrocyin)

White prisms (from methanol), m.p. 195–198°, tlc: R_f 0.60, HPLC: t_R 43.9 min. HR-FABMS: 545.3465 [$M^+ + 1$] calculated for $C_{32}H_{49}O_7$ (found 545.3469) EI-MS *m/z*: 498 (1.3) [$M^+ - 46$], 403 (1.4) [M^+ – side chain], 385 (2.7) [M^+ – side chain-H_2O], 367 (1.6), 166 (1.9), 111 (18.2), 96 (100), 87 (13.7) and 55 (9.5).

IR: ν_{max} 3427 (OH), 2939 (CH), 1697 (C=O), 1715 (C=O), 1654 (C=O), 1465, 1267, 1222, 1099, 1066, 1028 and 788.

^{1}H NMR, ^{13}C NMR, HMQC and HMBC data: see Table 2.

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