

Terpenoids from *Cleome droserifolia* (Forssk.) Del.

Hesham I. El-Askary

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt; e-mail heshamaskary@yahoo.com

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Abstract: A new diacetyl triterpene lactone, drosericarpone (**2**), was isolated from the hexane extract of the herb *Cleome droserifolia*, together with buchariol (**1**, a sesquiterpene oxide, isolated for the first time from *Cleome* species) and stigmasterol glucoside (**3**). The structures of **1-3** were identified by spectroscopic means.

Keywords: *Cleome droserifolia*, Cleomaceae, buchariol, drosericarpone

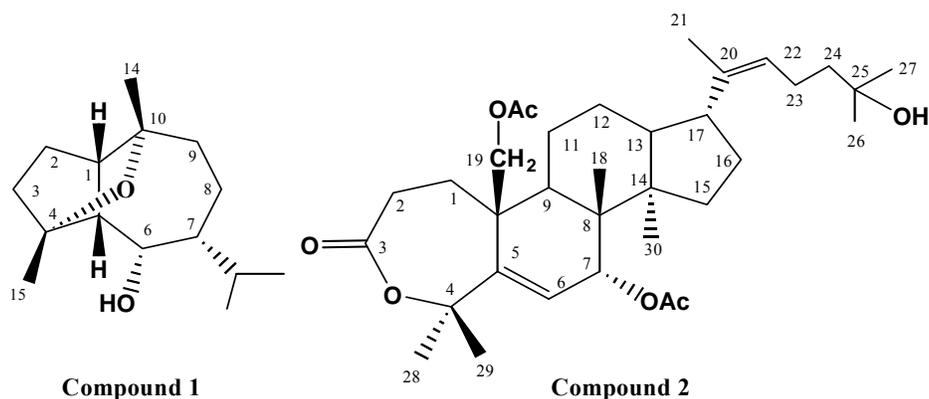
INTRODUCTION

Cleome droserifolia (Forssk.) Del. belongs to Family Cleomaceae [1,2]. *Cleome* species are generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation [3-6]. The dried herb of *C. droserifolia*, locally known as Samwah, Afein, Reeh-El-Bard [7], is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used by the Bedouins of the southern Sinai for the treatment of diabetes [8]. Several studies were carried out to confirm the hypoglycemic effect of the decoction of this herb [8-10].

Flavonoids [7,11-15] and sesquiterpenes (carotol and an unidentified one) were isolated from *C. droserifolia* [22]. To date, several dammarane triterpenes have been isolated from genus *Cleome*, viz. *C. amblyocarpa* Barr. Et Murb. [16] (syn. *C. arabica* auct. Non L. and *C. africana* Botsch [17,18] and *C. brachycarpa* Vahl ex. DC (Punwar) [19-21], but nothing has been reported on the isolation of dammarane triterpenes from *Cleome droserifolia*. Therefore, the following study was carried out to isolate and identify the constituents of the hexane extract of the plant.

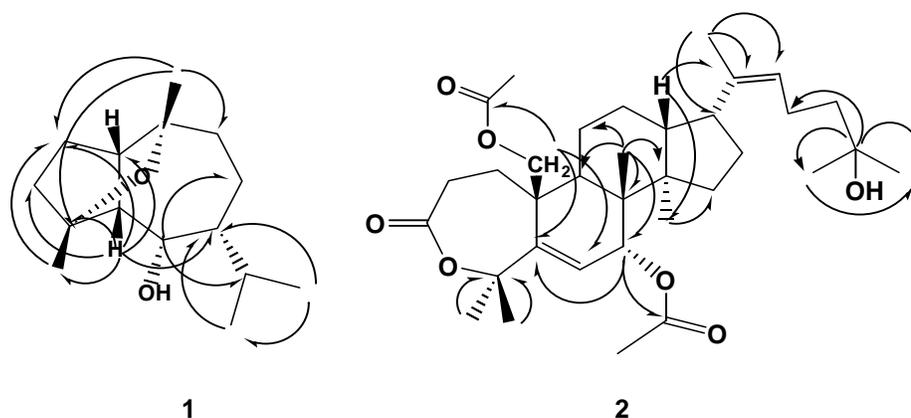
Results and Discussion:

The hexane fraction of the ethanolic extract of the powdered herb of *C. droserifolia* afforded three terpenoidal compounds **1-3**. The identification of these compounds was accomplished by examination of their spectral data (^1H -, ^{13}C -NMR, COSY, HMQC, HMBC and EIMS) and supported by comparison with published data of related compounds [16-30].



Compound **1**, $\text{C}_{15}\text{H}_{26}\text{O}_2$, EIMS, m/z 238 $[\text{M}]^+$, was identified as buchariol, previously isolated from the herb *Salvia bucharica*, by comparing its spectral data (Table 1) with that reported for this compound [24]. The complete assignment of the ^{13}C -NMR data of **1** was accomplished using 2D NMR spectra (HMQC and HMBC) and is reported here for the first time (Table 1 and Figure 1).

Figure 1. Long range correlations observed in the HMBC spectra of **1** and **2**



Compound **2** was isolated as an oily residue and had a molecular formula of $\text{C}_{34}\text{H}_{52}\text{O}_7$ (^{13}C -NMR data and DEPT experiment). Analysis of the ^1H - and ^{13}C NMR spectra of **2** with the aid of 2D-NMR spectra (^1H - ^1H COSY, HMQC and HMBC) revealed features characteristic of a triterpene unit containing a 7-membered lactone ring (IR, 1730 cm^{-1}) as compared with related compounds isolated from other *Cleome* species [20, 21]. The ^1H -NMR spectrum of **2** showed seven methyl singlet at δ_{H} 1.15, 1.26, 1.41, 1.47, 1.53, 1.56, and 2.18; which directly correlated with ^{13}C -NMR signals at δ_{C} 18.3, 29.2, 27.2, 23.1, 25.9, 16.2, and 31.5, respectively, an olefinic methyl (δ_{H} 2.1), nine methylenes [including one attached to an acetyl group (δ_{H} 4.56 *d* and 4.92 *d*, δ_{C} 61.8)], six methines [including one

oxymethine at δ_c 72.9 (δ_H 5.15 *d*) and two olefinic methines at δ_c 126.8 (δ_H 4.92 *t*) and 128.2 (δ_H 5.22 *d*), and ten quaternary carbons [including three carbonyl carbons (δ_c 177.6, 171.8 and 171.0), two olefinic carbons (δ_c 135.0 and 136.1), and a carbon bearing OH group at (δ_c 69.8)]. The spectra also revealed the presence of two acetyl groups (δ_H 2.0/ δ_c 22.8 and 2.03/ δ_c 21.2). HMBC correlations of **2** (Figure1) confirmed the gross structure of **2** to be a diacetyl triterpene lactone. The relative stereochemistry at C-7 was confirmed to be 7 β -H [7 α -H should appear as singlet or *br s* near δ_H 4.7 and H-6 should appear as *d* ($J=1.5$ Hz) near δ_H 5.9 as confirmed by ROESY [25], while H-7 appears at δ_H 5.15 as *d* ($J=10.5$ Hz) and H-6 appears at δ_H 5.22 as *d* ($J=10.5$ Hz)]. The chemical shifts of C-17 and C-21 were found comparable with those of a related compound with 17 β -H (C-17 δ_H 2.61 *dd* / δ_c 60.5 and C-21 δ_H 2.24/ δ_c 31.2) [26], suggested the β -orientation of H-17, compared with the data of related compounds with 17 α -H [25-29]. The stereochemistry of the double bond at C-20 (22) was proposed to be a *Z*-type; since the signal for C-21 was observed at δ_c 31.5 (C-21 of the *E*- type is usually observed near δ_c 13-15 [25]). From the above data, the structure of **2** was inferred to be as proposed and it was given the name *drosericarpone*. This compound is reported here for the first time from family *Cleomaceae* and from nature.

Compound **3**, C₃₅H₅₈O₆, was obtained as fine colorless needles (ethyl acetate), mp, 284°C, API-MS (positive ion mode) *m/z* 613.5 [(M+H)⁺Na]⁺. Its structure was identified as *stigmasterol glucoside* from comparison of its spectral data, (¹H- and ¹³C-NMR) with those previously reported [30].

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Experimental

General

M.p. was measured on a Gallekamp melting point apparatus and was uncorrected. 1D-¹H- (500MHz) and ¹³C-(125MHz) NMR spectra were recorded at 25°C using (benzene-*d*₆) as solvent and TMS as internal standard on a JEOL 500 Spectrophotometer. 2D-NMR spectra were obtained on a Bruker Avance DRX 400 Spectrophotometer. EI-MS was obtained on Shimadzu PQ-5000 (70 eV) and Bruker Autoflex (Bruker Daltonics, Germany) mass spectrometers. Atmospheric pressure ionization mass spectra (API-MS) were recorded using a PE SCIEX API III bimolecular mass analyzer. Silica gel 60 (70-230 mesh) and Silica gel RP-8 (both from Merck) were used for column chromatography and silica gel 60 H was employed for VLC technique. Centrifugal accelerated radial TLC was performed on a Chromatotron, model no.7924 (Harrison Research Inc. Palo, Alto, Calif., USA). TLC were conducted on precoated silica gel 60 F₂₅₄ plates (0.25 mm thickness, Merck), developed with the solvent system MeOH-CHCl₃ (5:95). The TLC plates were visualized by spraying with *p*-anisaldehyde reagent followed by heating at 110°C.

Plant material

Plant material was collected from the Suez-Cairo desert road, Egypt, in March 2002 and was kindly identified by Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen has been deposited in the herbarium of the Faculty of Pharmacy, Cairo University.

Isolation

The air-dried powdered herb of *C. droserifolia* (600 g) was extracted with 70% ethanol. The residue left after distillation of the solvent (75 g), was successively fractionated with hexane, chloroform and methanol. The hexane extract (3.2 g.) was chromatographed on a VLC column (14 cm L x 4 cm D) of silica gel H (50g), eluted with hexane, CHCl₃, EtOAc and MeOH, in increasing proportions until 5 % MeOH-EtOAc, in fractions, each of 200 mL. *Fraction A*: 980 mg, eluted with CHCl₃, showed a major violet spot with the spray reagent, R_f = 0.75. This fraction was further purified by CC (18 cm L x 3 cm D) on silica 60 eluted with 5% MeOH-CHCl₃, in fractions, each of 5 mL, which gave: *Fraction A-1* (combined frs.7-12, 760 mg) was further purified twice on a Chromatotron, eluted with 4% MeOH/CHCl₃, in fractions of 2 mL each, to give compound **1** (50 mg, oily residue, R_f = 0.35). *Fraction A-2* (frs. 40-66, 50 mg) was further purified twice on a SiO₂ CC, eluted with benzene, CHCl₃ → 20% MeOH-CHCl₃, to give a fraction, which was purified on CC/RP-18 (eluted with water/ MeOH) to give compound **2** (10 mg, oily residue, R_f = 0.52). *Fraction B* (290 mg, eluted with EtOAc) was further purified on a Chromatotron, eluted with 8% MeOH-CHCl₃, in fractions, each of 2 mL, to give compound **3** (14 mg, fine colorless needles, R_f = 0.6).

Buchariol (1), oily residue, C₁₅H₂₆O₂, [24]; EI -MS (*m/z*): 238 (M⁺, base peak), 220 (M⁺ - 18), 195 (M⁺ - 43, C₃H₇), 177 (M⁺ - H₂O - C₃H₇), 159 (M⁺ - 2H₂O - C₃H₇), 141, 81. ¹H- and ¹³C-NMR spectral data (CDCl₃) see Table 1.

Drosericarpone (2), oily residue, C₃₄H₅₂O₇; EI -MS (*m/z*): 446 [(M+H) - C₈H₁₅O]⁺, 388 [446 - Me₂CO]⁺, 328 (388 - CH₃COOH), 286, 268 (388 - 2 x CH₃COOH), 225, 135, 127, 121; IR ν_{max} (KBr) cm⁻¹: 3440 (OH), 1720, 1730 (carbonyl); ¹H- and ¹³C-NMR spectral data, see Table 1.

Stigmasterol glucoside (3), fine colorless needles (from ethyl acetate), mp. 284 °C; C₃₅H₅₈O₆; API-MS (positive ion mode) *m/z* 613.5 {(M+H)+ Na}⁺, 569.5 {(M+H)+ Na - C₃H₈, 44}⁺, 525.5 {(M+H)+ Na - 2 C₃H₈}⁺, 481.5 {(M+H)+ Na - 3 C₃H₈}⁺, 413.5 {(M+H) - 162}⁺, 393.5 (base peak), 349.5, 243, 295.5, 245.5, 133; IR ν_{max} (KBr) cm⁻¹: 3400 (OH), 2960-2850, 1640, 1465, 1380; ¹H-NMR (500 MHz, C₆D₆): δ 5.35 (1H, *t*, *J* = 4.7 & 1.7 Hz, H-6), 5.21 (1H, *dd*, *J* = 15.2 & 8.8 Hz, H-22), 5.05 (1H, *dd*, *J* = 15.2 & 8.8 Hz, H-23), 4.89 (1H, *d*, *J* = 7.9 Hz, H-1'), 4.42 (1H, *dd*, *J* = 2.4 & 11.7 Hz, H-6_a), 4.23 (1H, *dd*, *J* = 5.3 & 11.7 Hz, H-6_b), 4.12 (1H, *m*, H-3'), 4.08 (1H, *m*, H-4') 3.89 (1H, *t*, *J* = 7.9 & 8.8 Hz, H-2'), 3.84 (1H, *m*, H-5'), 2.62 (1H, *dd*, *J* = 2.6 & 12.3 Hz), 2.37 (1H, *t*, *J* = 11.3 & 11.5 Hz, H-7_a), 2.0 (1H, *m*, H-8), 1.9 (1H, *m*, H-7_b), 0.98 (3H, *d*, *J* = 6.4 Hz, Me-21), 0.92 (3H, *s*, Me-19), 0.88 (3H, *d*, *J* = 6.8 Hz, Me-26), 0.86 (3H, *d*, *J* = 6.8 Hz, Me-27), 0.81 (3H, *t*, *J* = 7 Hz, Me-29), 0.67 (3H, *s*, Me-18); ¹³C-NMR (125 MHz, C₆D₆): δ_c 37.4 (C-1, *t*) 28.5 (C-2, *t*), 78.1 (C-

3, *d*), 39.2 (C-4, *t*), 140.8 (C-5, *s*), 121.9 (C-6, *d*), 32.1 (C-7, *t*), 32.0 (C-8, *d*), 50.3 (C-9, *d*), 36.9 (C-10, *s*), 21.2 (C-11, *t*), 39.9 (C-12, *t*), 42.5 (C-13, *s*), 56.8 (C-14, *d*), 24.5 (C-15, *t*), 29.4 (C-16, *t*), 56.2 (C-17, *d*), 12.0 (C-18, *q*), 19.4 (C-19, *q*), 36.4 (C-20, *d*), 19.0 (C-21, *q*), 137.3 (C-22, *d*), 128.3 (C-23, *d*), 46.0 (C-24, *d*), 26.3 (C-25, *d*), 20.0 (C-26, *q*), 19.2 (C-27, *q*), 29.9 (C-28, *t*), 12.2 (C-29, *q*), sugar carbons, 102.4 (C-1', *d*), 75.0 (C-2', *d*), 78.2 (C-3', *d*), 71.5 (C-4', *d*), 78.1 (C-5', *d*), 62.7 (C-6', *t*).

Table 1. ^1H - and ^{13}C -NMR data of compound 1 and 2

Compound 1			Compound 2					
Position No.	δ_{C}	δ_{H}	Position No.	δ_{C}	δ_{H}	Position No.	δ_{C}	δ_{H}
1	53.3 <i>d</i>	2.34 <i>m</i>	1	34.6 <i>t</i>	2.05 <i>m</i> 2.46 <i>m</i>	16	29.3 <i>t</i>	1.36 <i>m</i> 1.65 <i>m</i>
2	23.8 <i>t</i>	1.52 <i>m</i> 1.57 <i>m</i>	2	24.6 <i>t</i>	2.26 <i>m</i>	17	53.7 <i>d</i>	2.63 <i>m</i>
3	37.5 <i>t</i>	1.39 <i>m</i> 1.75 <i>m</i>	3	177.6 <i>s</i>	-	18	18.3 <i>q</i>	1.15 <i>s</i> 3H
4	74.3 <i>s</i>	--	4	84.8 <i>s</i>	-	19	61.8	4.56 <i>d</i> 4.92 <i>d</i> (12.5 Hz)
5	68.1 <i>d</i>	2.33 <i>m</i>	5	136.1 <i>s</i>	-	20	135.0 <i>s</i>	-
6	75.9 <i>d</i>	4.00 (1H,br <i>dd</i> (1.5 Hz))	6	128.2 <i>d</i>	5.22 <i>d</i> (10.5 Hz)	21	31.5 <i>q</i>	2.18 <i>s</i> 3H
7	38.5 <i>t</i>	1.38 <i>m</i>	7	72.9 <i>d</i>	5.15 <i>d</i> (10.5 Hz)	22	126.8 <i>d</i>	4.92 <i>t</i>
8	20.2 <i>t</i>	1.55 <i>m</i> 1.80 <i>m</i>	8	49.7 <i>d</i>	-	23	44.6 <i>t</i>	2.67 <i>m</i> 2H
9	48.1 <i>t</i>	2.12 <i>m</i> 2.21 <i>m</i>	9	40.4 <i>d</i>	1.30 <i>m</i>	24	44.6 <i>t</i>	2.67 <i>m</i> 2H
10	74.4 <i>s</i>	--	10	45.0 <i>s</i>	--	25	69.8 <i>s</i>	-
11	32.6 <i>d</i>	1.72 <i>m</i>	11	37.2 <i>t</i>	1.28 <i>m</i> 1.74 <i>m</i>	26	29.2 <i>q</i>	1.26 <i>s</i> 3H
12	21.0 <i>q</i>	0.95 3H <i>d</i> (6.8 Hz)	12	26.1 <i>t</i>	1.37 <i>m</i> 1.54 <i>m</i>	27	27.2 <i>q</i>	1.41 <i>s</i> 3H
13	21.0 <i>q</i>	0.94 3H <i>d</i> (6.8 Hz)	13	40.4 <i>d</i>	1.68 <i>m</i>	28	23.1 <i>q</i>	1.47 <i>s</i> 3H
14	25.7 <i>q</i>	1.42 <i>s</i> 3H	14	49.7 <i>s</i>	--	29	25.9 <i>q</i>	1.53 <i>s</i> 3H
15	21.9 <i>q</i>	1.18 <i>s</i> 3H	15	37.8 <i>t</i>	2.08 <i>m</i> 2.23 <i>m</i>	30	16.2 <i>q</i>	1.56 <i>s</i> 3H

Table 1. Cont.

Compound 1			Compound 2					
Position No.	δ_c	δ_H	Position No.	δ_c	δ_H	Position No.	δ_c	δ_H
			Acetyl at C-7	171.0 s 22.8 q	- 2.00 s 3H	Acetyl at C-19	171.8 s 21.2 q	- 2.03 s 3H

Assignments were verified using the 2D-NMR (^1H - ^1H COSY, HMQC and HMBC) experiments, and multiplicity was determined by DEPT experiments and J values are given in parenthesis.

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Sample Availability: Contact the author.