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-, 13C-NMR, COSY, HMQC, HMBC and EIMS) and supported by comparison with published data of related compounds [16-30].

Compound 1, C_{13}H_{26}O_{2}, EIMS, m/z 238 [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 13C-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).

![Compound 1 and Compound 2](image)

**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 C_{34}H_{52}O_{7} (13C-NMR data and DEPT experiment). Analysis of the 1H- and 13C 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 13C-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 7β-H [7α-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 17β-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 17α-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 13.5 (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 Cleomeaceae and from nature.

Compound 3, C_{35}H_{58}O_{6}, 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-d6) 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 F_{254} plates (0.25 mm thickness, Merck), developed with the solvent system MeOH-CHCl_{3} (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, R₇ = 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, each of 2 mL, to give compound 1 (50 mg, oily residue, R₇ = 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, R₇ = 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, R₇ = 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 – CH₂COOH), 286, 268 (388 – 2 x CH₂COOH), 225, 135, 127, 121; IR νmax (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 – C₃H₇}, 444, 525.5 {(M+H)+ Na – 2 C₃H₇}, 481.5 {(M+H)+ Na– 3 C₃H₇}⁺, 413.5 {(M+H) - 162}⁺, 393.5 (base peak), 349.5, 243, 295.5, 245.5, 133; IR νmax (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-6), 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-1), 4.32 (1H, dd, J = 5.3 & 11.7 Hz, H-6'), 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-7a), 2.0 (1H, m, H-8), 1.9 (1H, m, H-7b), 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 37.4 (C-1), 28.5 (C-2), 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. $^1$H- and $^{13}$C-NMR data of compound 1 and 2

| Compound 1 | Compound 2 |
|------------|------------|
| Position No. | $\delta_c$ | $\delta_H$ | Position No. | $\delta_c$ | $\delta_H$ | Position No. | $\delta_c$ | $\delta_H$ |
| 1 | 53.3 d | 2.34 m | 1 | 34.6 t | 2.05 m | 2.46 m | 16 | 29.3 t | 1.36 m |
| 2 | 23.8 t | 1.52 m | 2 | 24.6 t | 2.26 m | | 17 | 53.7 d | 2.63 m |
| 3 | 37.5 t | 1.39 m | 3 | 177.6 s | - | | 18 | 18.3 q | 1.15 s 3H |
| 4 | 74.3 s | - | 4 | 84.8 s | - | | 19 | 61.8 | 4.56 d |
| | | | | | | | | 4.92 d |
| | | | | | | | | (12.5 Hz) |
| 5 | 68.1 d | 2.33 m | 5 | 136.1 s | - | | 20 | 135.0 s | - |
| 6 | 75.9 d | 4.00 (1H,br dd (1.5 Hz) | 6 | 128.2 d | 5.22 d | (10.5 Hz) | 21 | 31.5 q | 2.18 s 3H |
| 7 | 38.5 t | 1.38 m | 7 | 72.9 d | 5.15 d | (10.5 Hz) | 22 | 126.8 d | 4.92 t |
| 8 | 20.2 t | 1.55 m | 8 | 49.7 d | - | | 23 | 44.6 t | 2.67 m |
| | 1.80 m | | | | | | | 2H |
| 9 | 48.1 t | 2.12 m | 9 | 40.4 d | 1.30 m | | 24 | 44.6 t | 2.67 m |
| | 2.21 m | | | | | | | 2H |
| 10 | 74.4 s | - | 10 | 45.0 s | - | | 25 | 69.8 s | - |
| 11 | 32.6 d | 1.72 m | 11 | 37.2 t | 1.28 m | 1.74 m | 26 | 29.2 q | 1.26 s 3H |
| 12 | 21.0 q | 0.95 3H d (6.8 Hz) | 12 | 26.1 t | 1.37 m | 1.54 m | 27 | 27.2 q | 1.41 s 3H |
| 13 | 21.0 q | 0.943H d (6.8 Hz) | 13 | 40.4 d | 1.68 m | | 28 | 23.1 q | 1.47 s 3H |
| 14 | 25.7 q | 1.42 s 3H | 14 | 49.7 s | - | | 29 | 25.9 q | 1.53 s 3H |
| 15 | 21.9 q | 1.18 s 3H | 15 | 37.8 t | 2.08 m | 2.23 m | 30 | 16.2 q | 1.56 s 3H |
Table 1. Cont.

| Compound 1 | Compound 2 |
|------------|------------|
| **Position** | **δ\text{c}** | **δ\text{H}** | **Position** | **δ\text{c}** | **δ\text{H}** |
| **No.** | | | | | |
| 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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