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Cytotoxic *neo*-clerodane diterpenes from *Stachys aegyptiaca*

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ABSTRACT

Two new *E/Z neo*-clerodane diterpene isomers, trivially named stachaegyptin D (**1**) and E (**2**), together with known compounds, stachysolon monoacetate (**3**) and stachysolon diacetate (**4**), were isolated from an organic-solvent extract of the medicinal herb *Stachys aegyptiaca*. Structures were elucidated by a combination of spectroscopic methods, including HREIMS, ^{1}H, ^{13}C, DEPT, and 2D NMR analysis, as well as, first time X-ray analysis for stachysolone (**3**). All isolated metabolites showed some activity against the human hepatocellular cell line, HepG2. Compound **4** was the most active with an IC_{50} of 59.5 μM.

1. Introduction

Geographically, the Sinai Peninsula has a unique environmental ecosystem, giving rise to significant medicinal-plant biodiversity that draws ecologists, taxonomists and phytochemists from around the world. *Stachys* L. is well represented in the Sinai and is one of the largest genera in the Lamiaceae family comprising. It has over 300 species distributed in temperate and tropical regions throughout the world, except for Australia and New Zealand (Tundis et al., 2014). Select *Stachys* species showed anti-inflammatory, cytotoxic, antitoxic, antibacterial and antioxidant activities (Tundis et al., 2014). Previous phytochemical studies of *Stachys aegyptiaca* Pers, locally named Qourtom, reported different constituents including essential oils (Salimi et al., 2010; Halim et al., 1991) diterpenes (Hegazy et al., 2017; Mohamed and Mohamed, 2014; Melek et al., 1992), and flavonoids (Hegazy et al., 2017; El-Desoky et al., 2007; Sharaf, 1998; El-Ansari et al., 1991, 1995).

Herein, four *neo*-clerodane diterpenes, including two new compounds that were isolated from the aerial parts of *S. aegyptiaca* (Fig. 1). The structures of the isolated diterpenoids (**1-4**) were determined by spectroscopic analyses. Additionally, antiproliferative activity was calculated based on compound behavior based on a bioassay using a human hepatocellular carcinoma cell lines (HepG2).

2. Results and discussion

2.1. Structure elucidation of isolated diterpenoids

The crude methylene chloride/methanol (1:1) extract of the air-dried aerial parts of *S. aegyptiaca* was subjected to normal and reverse phase chromatography to afford new compounds 1–2, in addition to known compounds 3–4 (Fig. 1).

Compound **1** was obtained as a colorless oil with a negative optical rotation $[\alpha]_D^{25}$ −55.0 (c 0.01, MeOH). HR-FAB-MS analysis showed a molecular ion peak at m/z 385.2358 $[M + Na]^+$ (calcd. for $C_{22}H_{34}O_4Na$, 385.2355), corresponding to a molecular formula of $C_{22}H_{34}O_4$. The IR spectrum showed characteristic bands for a hydroxyl at 3410 cm^{-1} and carbonyl groups at 1731 and 1641 cm^{-1}. The ^{13}C NMR spectrum showed 20 signals (Table 1), which were further differentiated by DEPT to 4 methyls, 6 methylenes (2 olefinic), 4 methines (1 oxygenated, 2 olefinic), and 5 quaternary carbons (1 keto and 2 olefinic). The appearance of a hydroxylated carbon methine doublet proton at δ_H 4.03 (brd, J = 2.7 Hz, H-7) correlate with methyl, methylene and methine carbons at δ_C 12.5, 41.5 and 38.9, respectively in the HMBC spectrum. Additionally, four methyl groups at δ_H 1.86 (s), δ_H 1.35 (s), δ_H 1.03 (s) and δ_H 0.98 (d, J = 7.0 Hz) were observed in the ^{1}H NMR spectrum (Table 1). Six degrees of unsaturation were deduced suggesting a bicyclic diterpene skeleton and these spectroscopic data were consistent with a previously reported *neo*-clerodane type diterpene (Adinolfi et al., 1984). Two-dimensional COSY, HMQC and

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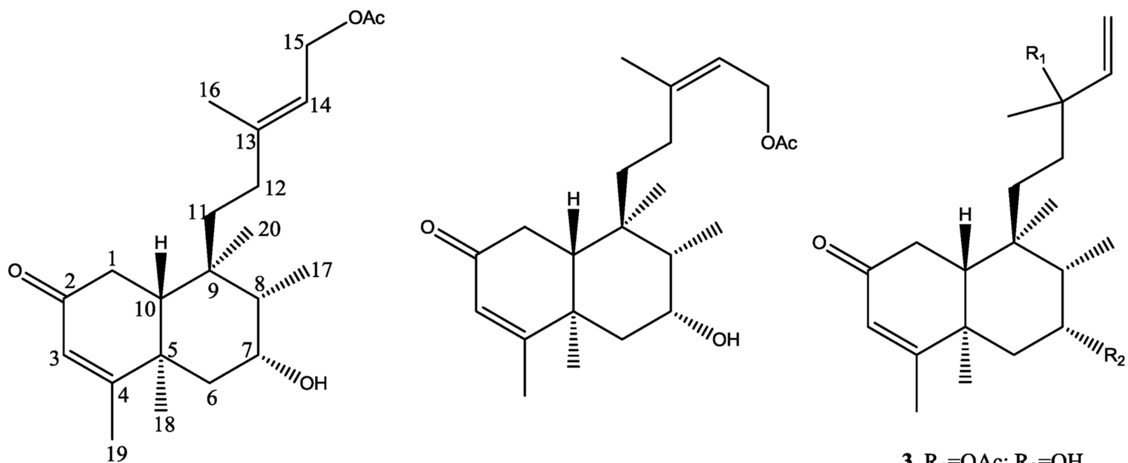

1 (*E*) **2** (*Z*) **3** R_1=OAc; R_2=OH **4** R_1=OAc; R_2=OAc

Fig. 1. Structures of the isolated diterpenes from *S. aegyptiaca.*

Table 1
^{1}H and ^{13}C NMR Spectral Data of stachaegyptin D (**1**) and E (**2**) (600 MHz).

No	Stachaegyptin D (1)		Stachaegyptin E (2)	
	δ_H (*J* in Hz)	δ_C; Type	δ_H (*J* in Hz)	δ_C; Type
1	2.29 dd (3.4, 15.0) 2.39 dd (13.7, 17.0)	34.8; CH_2	2.33 dd (3.4, 15.0) 2.43 dd (14.0, 17.0)	34.9; CH_2
2	———	200.4; C	———	200.0; C
3	5.63 brs	125.0; CH	5.66 brs	125.0; CH
4	———	173.1; C	———	172.8; C
5	———	38.2; C	———	38.4; C
6	1.45 dd (3.4, 14.0) 2.14 dd (2.7, 14.0)	41.5; CH_2	1.49 dd (3.4, 14.0) 2.16 dd (3.4, 14.0)	41.5; CH_2
7	4.03 brd (2.7)	73.1; CH	4.06 brd (3.4)	73.1; CH
8	1.52 m	38.9; CH	1.58 m	38.9; CH
9	———	39.5; C	———	39.4; C
10	1.88* m	45.9; CH	1.94* m	45.9; CH
11	1.31* m	36.8; CH_2	1.24* m	37.3; CH_2
12	1.73* m	32.6; CH_2	1.68* m	25.4; CH_2
13	———	142.1; C	———	143.0; C
14	5.25 brt (6.9)	118.4; CH	5.27 br t (7.5)	119.0; CH
15	4.49 d (7.5)	61.4; CH_2	4.47 d (7.5)	60.7; CH_2
16	1.62 s	16.9; CH_3	1.71 s	19.1; CH_3
17	0.98 d (7.0)	12.5; CH_3	1.02 d (7.0)	12.4; CH_3
18	1.86 s	19.1; CH_3	1.88 s	19.5; CH_3
19	1.35 s	20.2; CH_3	1.37 s	21.1; CH_3
20	1.03 s	19.6; CH_3	1.03 s	20.2; CH_3
OAc	1.99 s	21.1; CH_3 171.2; C	2.01 s	23.7; CH_3 171.1

Assignments were based on HMBC, HSQC, COSY and DEPT experiments.
*Overlapped proton NMR signals.

HMBC signals matched signals from published analogues (Hegazy et al., 2017; Mohamed and Mohamed, 2014).

Based on similar *neo*-clerodane type diterpene structures, a characteristic oxygenated H-7 was identified at δ_H 4.03 (br d, J = 2.7) (Adinolfi et al., 1984) and using this as a point of reference, H-7 allowed for the assignment of H_2-6 (δ_H 1.45/2.14), and H-8 (δ_H 1.52) through DQF-COSY analysis. The olefinic signal at δ_H 5.63 (brs) correlated via HMBC with a keto group at δ_C 200.4 and a quarternary olefinic signal at δ_C 173.1 that was expected since an endocyclic double bond between C-3/C-4 is often present with *neo*-clerodane type diterpenes (Melek et al., 1992; Adinolfi et al., 1984; Popa and Orgiyan, 1974) (Fig. 2). A methyl signal at δ_H 1.03 (s) correlated via HMBC with C-9 (δ_C 39.5), C-8 (δ_C 38.9), C-10 (δ_C 45.9) and C-11 (δ_C 36.8) indicating the location of the side change at C-9. Additionally, the proton signal at δ_H 1.31 (H_2-11) correlated with δ_H 1.73 in DQF-COSY that allowed for the assignment at H_2-12. The downfield quaternary olefinic

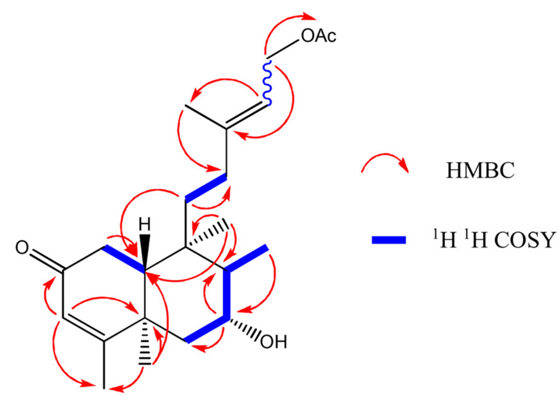

Fig. 2. Observed COSY and HMBC correlations of compounds **1** (***E***) and **2** (***Z***).

signal at δ_C 142.1 was assigned to C-13 that correlated via HMBC with H-12 (δ_H 1.73 m), H-14 (δ_H 5.25), H-15 (δ_H 4.49) and H-16 (δ_H 1.62) indicating the presence of a double bond as part of a side chain system. The presence of an acetylated methylene (H_2-15) at δ_C 61.4 was confirmed by HMBC correlations of H-15 (δ_H 4.49, d, J = 7.5 Hz) with the quaternary olefinic carbon C-13 at δ_C 142.1 and the acetyl keto group at δ_C 171.2 (Fig. 2). DQF-COSY showed correlation between H-10 (δ_H 1.88 m) and methylene protons (H_2-1) at δ_H 2.29 (dd, J = 3.4, 15.0 Hz) and 2.39 (dd, J = 14.0, 17.0 Hz), allowing for the assignment of H-1 (Fig. 2).

The relative β-configuration H-7 was deduced based on biogenetic precedent and was consistent with previously reported NMR chemical shift data for similar *neo*-clerodane type diterpene (Hegazy et al., 2017; Melek et al., 1992; Adinolfi et al., 1984; Popa and Orgiyan, 1974). NOE correlations of H-8 at δ_H 1.52 m/ H-7β at δ_H 4.03 (brd, J = 2.7 Hz), and H-7/ H-10 at δ_H 1.88 (m) indicated that these protons were all on the same face in the β-configuration (Fig. 3). Additionally, H_3-17 correlated with δ_H 1.86 (s, H_3-18) and 1.03 (s, H_3-20) establishing that it is on the opposite α-face. The NMR data of **1** was consistent with those of the known compound; stachysolon monoacetate (**3**) (Adinolfi et al., 1984). For stachysolon monoacetate (**3**), data was deduced by X-ray crystallography analysis (Adinolfi et al., 1984) (Fig. 4). Compounds **1** and **3** exhibit the same stereochemistry (Fig. 4). A NOESY experiment established that the vanylic proton at H-14 (δ_H 5.2 5) correlates with methylene protons at H-15 (δ_H 4.49 d, J = 7.5 Hz) that indicated that the Δ^{13} double bond has a *E* configuration. Therefore **1** is assigned as 15-acetoxy-2-oxo-*neo*-cleroda-3,13(*E*)-dien-7α-ol (Stachaegyptin D).

Compound **2** was isolated as colorless oil with a negative optical