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New Rearranged Abietane Diterpenoids from the Roots of *Salvia aegyptiaca* L. Growing in Egypt

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Abstract – Two new abietane diterpenes with rearranged skeleton; aegyptinol and aegyptinone C; have been isolated and identified for the first time from the anti-microbial petroleum ether extract of the roots of *Salvia aegyptiaca* L. Their chemical structures have been elucidated by interpretation of the detailed 1D- and 2D-NMR spectra, as well as other spectroscopic tools. In addition, full assignment of ^{13}C- NMR of aegyptinone B was also conducted for the first time.

Keywords – *Salvia aegyptiaca* L., Lamiaceae, abietane diterpenoids with rearranged skeleton, aegyptinone C, aegyptinol

Introduction

Plants of the genus *Salvia* (Lamiaceae) have been extensively studied in the last few decades. Their contents of tanshinones and royleanones are the subject of great interest due to their anti-bacterial (Topcu and Ulubelen, 1996), anti-oxidative (Cuvelier *et al.*, 1996), anti-viral (Tada *et al.*, 1994) anti-tubercular (Ulubelen *et al.*, 1997) activities. Genus *Salvia* is represented in Egypt by 10 species of which *S. verbenaca*, *S. lanigera* and *S. aegyptiaca* are the most common (Tackholm, 1974). Our previous work (Sabri *et al.*, 1989) on the anti-microbial petroleum ether extract of the roots of *Salvia aegyptiaca*, resulted in the isolation of two new abietane diterpenoids with rearranged skeleton related to tanshinone-type namely, aegyptinones A and B. In a continuation of this study, we now report the isolation and structure elucidation of two more structurally related abietane diterpenoids, aegyptinone C and aegyptinol. In addition, ^{13}C-NMR spectrum of aegyptinone B (Sabri *et al.*, 1989) was revised and completed using 2D-NMR spectra.

Experimental

Salvia aegyptiaca L. was collected in April 2000 from wild plants growing wildly in Marsa Matrouh, 160 km west of Alexandria. The plant was previously identified by Professor L. Bolous, of the National Research Center, Cairo, Egypt. Voucher specimens are also deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University.

General – ^{1}H-NMR (300MHz) and ^{13}C-NMR (75MHz) were recorded on Bruker AMX300 instrument, samples were dissolved in CD_3OD. UV spectra were recorded on Perkin Elmer 550 spectrophotometer. IR spectra were recorded on Perkin Elmer 1600 spectrophotometer. EIMS were recorded on Finnigan SSQ/7000,70ev. Silica gel (70-230 mesh, Merck) for column chromatography. TLC was carried out on pre-coated plates (Silica gel 60 F-245, Merck) with adsorbent layer thickness 0.25 mm. Detection was achieved by spraying with anisaldehyde/H_2SO_4 and heating at 105°C for 5 min.

Extraction and Isolation – The air-dried powdered roots of *S. aegyptiaca* (600 g) were extracted exhaustively with petrol in Soxhlet apparatus. The residue left after the evaporation of the solvent (5 g) was chromatographed over a column of silica gel (250 g). Elution was performed with a mixture of petrol-$CHCl_3$ (80:20) with gradual increase of $CHCl_3$. 40 fractions; 250 ml each; were collected, screened by TLC and similar fractions were combined. Fractions 14-15 (40% $CHCl_3$) and 26-29 (70% $CHCl_3$) afforded the previously isolated aegyptinones A and B respectively. Preparative TLC of fraction 22 (60% $CHCl_3$), using petrol: EtOAc (95:5) as a solvent system for a double run, afforded 5 mg of dark orange residue designated as aegyptinone C. while preparative TLC of fractions 31-32 (80% $CHCl_3$), using petrol:EtOAc (90:10) for multiple runs, afforded 8 mg of a pale yellow residue designated as aegyptinol.

Aegyptinone C **1** (5 mg): dark orange residue, Uv λ_{max} (MeOH): 226,284,327,371,420nm. IRγ_{max}(KBr): 3439,2969,

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Table 1. ^{1}H- and ^{13}C-NMR spectral data of compounds **1** and **2** and ^{13}C-NMR spectral data of eagyptionone B

Atom no.	**1** (CD_3OD)		**2** (CD_3OD)		**3** (CD_3OD)
	δ^{1}H (Hz)	δ^{13}C	δ^{1}H (Hz)	δ^{13}C	δ^{13}C
1	2.74t(6.5)	27.89t	2.72t(6.3)	27.27t	28.45t
2	1.87m	18.65t	1.92m	19.91t	20.19t
3	1.65m	37.39t	1.78m	39.89t	37.93t
4		34.39s		35.88s	34.95s
5		141.39s		134.10s	141.97s
6		152.07s		153.55s	152.60s
7	8.08s	123.09d	7.55s	130.61d	123.69d
8		128.78s		129.01s	132.09s
9		124.35s		121.83s	125.04s
10		141.10s		148.70s	140.77s
11		185.39s		172.45s	185.74s
12		157.57s		168.25s	153.73s
12-OH	7.79s				
13		119.70s		113.39s	122.71s
14		183.12s		164.10s	183.74s
15	3.36hep.(7.2)	30.14d	3.43m	39.76d	33.19s
16:	1.29d(7.2)	18.63q		79.97t	64.57t
16α			α:4.23dd(9.3,5.0)		
16β			β:4.70t(9.3)		
17	1.29d(7.2)	18.63q	1.36d(6.8)	20.34q	13.93q
18	1.33s	29.06q	1.35s	32.12q	30.96q
19	2.33s	29.06q	1.35s	32.12q	30.96q
20	2.66s	15.21q	5.25s	70.29t	15.79q

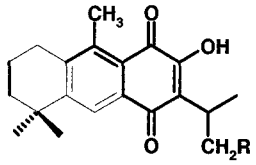
2932,1662,1644,1626,1570,1334,1313 cm^{-1}. EIMS, *m/z* (rel. int.): 312(10.9), 297(11.9), 245(75.2), 229(26.6), 185(21.0), 165(12.8), 149(32.8), 141(22.2), 128(32.7), 115(21.5), 95 (19.4), 84(92.8), 66(80.2), 55(100). ^{1}H- and ^{13}C-NMR results are summarized in Table 1.

Aegyptinol **2** (8 mg): pale yellow residue, UVλ_{max} (MeOH): 224, 240,282,315nm. IRγ_{max} (KBr): 3448br, 3080, 2961, 2884, 2872, 1646, 1607, 1562, 1485, 1478, 1446, 1362, 1237, 1142 cm^{-1}. EIMS, *m/z* (rel.int.): 328(11.6), 314 (13.3), 298(38.3), 283((33.3), 276(30.2), 245(60.0), 229 (28.4), 201(25.3), 185(45.0), 141(42.0), 128(45.1), 91(55.0), 79(80.2), 69(90.1), 55(100). ^{1}H- and ^{13}C-NMR results are summarized in Table 1.

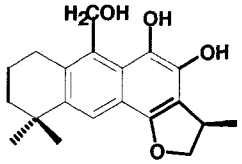
Results and Discussion

The molecular formula of compound **1** was deduced to be $C_{20}H_{24}O_3$, based on different spectral evidence. EIMS showed the molecular ion peak at m/z 312. ^{1}H-NMR was integrated for 24 protons, while ^{13}C-NMR revealed the presence of 18 resolved signals representing 20 carbons. The UV absorptions of compound **1** at 327, 371 and 420 nm indicted a highly conjugated system. The IR of compound **1** showed the presence of chelated and non-chelated quinonoidal carbonyls at 1662, 1644 and 1626 cm^{-1}. This was confirmed by ^{13}C-NMR spectrum through the appearance of two singlets at δ 185.39 and 183.12. Compound **1** showed NMR spectra with certain similarities to aegyptinone B. ^{1}H-NMR spectrum of compound **1** showed the presence of a *gem*-dimethyl, an aromatic methyl singlet and a benzenoid proton singlet located *peri* to a carbonyl group appearing at δ 1.33, 2.66 and 8.08, respectively. The only difference observed is the disappearance of the hydroxy-methylene group and the appearance of a methyl group in compound **1**. This again was supported by observing the molecular ion peak at *m/z* 312 i.e 16 mass units less than that of aegyptinone B. Different 2D-NMR spectra allowed full assignment of the chemical structure of (**1**), to which the trival name aegyptinone C was given.

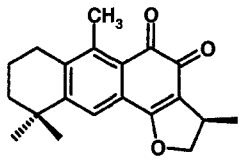
Different spectral data of compound **2** indicated an abietane diterpenoid with a rearranged skeleton having the molecular ion peak observed at *m/z* 328, while ^{13}C-NMR revealed the presence of 20 resolved carbon signals. DEPT-135° experiment indicated these carbons to be: $3CH_3$; $5CH_2$ of which two are oxygenated; 2CH and 10 aromatic carbons. Similar to aegyptinone A, ^{1}H-NMR spectrum of compound **2** confirmed the presence of a dihydromethyl-furan moiety, through the appearance of a methyl doublet at δ 1.36 (J=6.8Hz) coupled to a methine multiplet at δ 3.34, which in turn coupled to α- and β-methylene protons appearing at δ 4.23 (dd, J=9.3, 5.0Hz) and δ 4.70 (t, J=9.3Hz). HMQC experiment showed their corresponding carbons to be at δ 20.34, 39.97 and 79.97 respectively. The IR spectrum of compound **2** was free from carbonyl



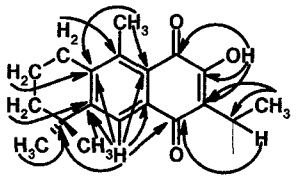
1 Aegyptinone C R=H
Aegyptinone B R=OH



2 Aegyptinol



Aegyptinone A



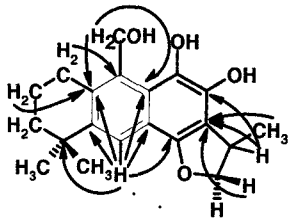


Fig. 1. The most important $^{1}H,^{13}C$-correlations as observed from the HMBC spectrum.

absorption and showed the appearance of a broad absorption band centered at 3440 cm^{-1} indicated the presence of OH group(s), while its phenolic nature was confirmed by $FeCl_3$ test solution. Unlike aegyptinone A, ^{13}C-NMR spectra were free from quinonoidal carbonyls, instead it indicated the presence of two phenolic hydroxyls through the appearance of two carbon signals at δ 172.45, 168.25. In addition, ^{1}H-NMR spectrum showed the presence of a singlet signal at δ 5.25, with its corresponding carbon signal appearing at δ 70.29, due to a hydroxy-methylene group at position 10. ^{1}H-NMR spectrum showed also two benzylic protons at δ 2.73 (t, J=6.3Hz) due to C-1 methylene and a gem-dimethyl group at δ 1.35 (s, 6H). Full assignment of protons and carbons was made possible by extensive study of HMQC and HMBC experiments. The absolute configuration for (**1**) at C-15(R) is assumed to be the same as that of aegyptinones A and B and the other structurally related cryptotanshinones (Tomita and Ikeshiro, 1987). Compound **2** was given the trival name aegyptinol. Figure 1 showed the most important ^{1}H, ^{13}C-correlations for aegyptinone C and aegyptinol, as observed from HMBC experiments.

It is important to mention that, the complete ^{13}C-NMR spectrum of aegyptinone B is carried out and reported in this paper for the first time, as the quaternary carbons could not be observed in our previous work (Sabri *et al*, 1989). In addition, extensive study of 2D-NMR spectra (HMQC and HMBC) has lead to the correct assignment of the methylene protons at positions 1 and 2 to their corresponding carbons.

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