Acknowledgment. We are grateful to Dr. H.-P. Husson of CNRS for the spectra (^1H and ^{13}C NMR and mass) of (-)-monomorine I and also to Dr. J. W. Daly of NIH and Professor T. Tokuyama of Osaka City University for the spectra (^1H and ^{13}C NMR and mass) of natural indolizidine 223AB. We thank Dr. H. Shindo of Tokyo College of Pharmacy for helpful discussions in the estimation of the energy barrier in the nitrogen inversion.

Two New Rearranged Abietane Diterpene Quinones from *Salvia aegyptiaca* L.

Nawal N. Sabri,* Amina A. Abou-Donia, Nabila M. Ghazy, Aya M. Assad, and Abdalla M. El-Lakany

Department of Pharmacognosy, Faculty of Pharmacy, Alexanderia University, Egypt

Dale R. Sanson, Hanna Gracz, Charles L. Barnes, Elmer O. Schlemper, and Michael S. Tempesta*

Department of Chemistry, University of Missouri, Columbia, Missouri 65211

Received February 22, 1989

Two novel diterpene quinones with rearranged abietane skeletons, aegyptinones A (1) and B (2), have been isolated from the antimicrobial petrol extract of *Salvia aegyptiaca* L. roots. Their structures have been established primarily by interpretation of detailed NMR data obtained from the experiments COSY, NOESY, INAPT, and QUAT, as well as other spectroscopic evidence. The structure of 1 was further confirmed by single-crystal X-ray analysis.

Salvia plants have been extensively studied in the last few years. The reported tanshinone and royleanone diterpene quinones are the subject of great interest, due to their antimicrobial and/or anticancer properties.[1-3] In a screening of Egyptian *Salvia* species for antimicrobial activity, we found that most were inhibitors of a variety of microorganisms. This activity prompted us to explore their chemistry.[4-8] In a continuation of these studies, we now report[9] the isolation and characterization of two novel structurally related, rearranged abietane diterpene quinones from the roots of *Salvia aegyptiaca* L., one of the common plants hitherto unexamined.

The petrol extract of *S. aegyptiaca* roots showed potent inhibitory activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. When this extract was chromatographed on silica gel column, followed by preparative TLC, it afforded two major colored crystalline components, 1 and 2. Physiochemical properties of these compounds indicated that both were homologous components, related to the tanshinones.[1,3,10]

Table I. ^1H (δ, Multiplicity, Coupling Constants in Hertz) and ^{13}C NMR (δ, Multiplicity) Assignments at 300 and 75 MHz in $CDCl_3$, Respectively, of Aegyptinones A (1) and B (2)

	1		2	
atom no.	^1H	^{13}C	^1H	^{13}C
1	2.72 b t (6.3)	19.1 t	2.75 b t (6.3)	19.6 t
2	1.87 m	28.5 t	1.87 m	29.0 t
3	1.68 m	37.7 t	1.68 m	38.2 t
4	-	34.9 s	-	n.o.[a]
5	-	141.2 s	-	n.o.
6	-	152.6 s	-	n.o.
7	7.58 s	121.6 d	8.12 s	125.7 d
8	-	125.5 s	-	n.o.
9	-	126.2 s	-	n.o.
10	-	144.0 s	-	n.o.
11	-	184.5 s	-	n.o.
12	-	176.2 s	-	n.o.
13	-	118.2 s	-	n.o.
14	-	171.0 s	-	n.o.
15	3.61 m	34.6 d	3.50 m	33.5 d
16α	4.39 dd (8.7, 5.5)	81.3 t	3.89 m	66.0 t
16β	4.90 t (8.7)		3.95 m	
17	1.38 d (6.8)	18.8 q	1.33 d (6.7)	15.0 q
18	1.35 s	31.9 q	1.35 s	31.4 q
19	1.35 s	31.9 q	1.35 s	31.4 q
20	2.60 s	16.6 q	2.60 s	17.2 q

[a] N.o. = not observed.

Aegyptinone A (1), $C_{20}H_{22}O_3$ (HRMS), was obtained as dark-orange prisms, mp 136 °C. The evidence for structure 1 is as follows: The UV and IR absorption spectra were consistent of an *o*-naphthoquinone chromophore[10] and somewhat similar to those of cryptotanshinone (3).[3] The MS showed mass peaks at m/z 178, 165, and 152, typical of tanshinone fragmentations.[3] The ^1H NMR assignments were made based on COSY and NOE experiments. The

(1) Fang, C.; Chang, P.; Hsu, T. *Hua Husueb Hsueb* Pao **1978**, *34*, 197; *Chem. Absr.* **1978**, *88*, 177078z.
(2) Kupchan, S. M.; Karim, A.; Marcks, C. *J. Am. Chem. Soc.* **1968**, *90*, 5923-5924.
(3) Ontisuka, M.; F-Ujiu, M.; Shinma, N.; Maruyama, H. B. *Chem. Pharm. Bull.* **1983**, *31*, 1670-1675.
(4) Haddad, D. Y.; Saleh, M. R. I.; Sabri, N. N. *J. Pharm. Sci., U.A.R.* **1962**, *7*, 215-222.
(5) Saleh, M. R. I.; Sabri, N. N.; Haddad, D. Y. *J. Pharm. Sci. U.A.R.* **1964**, *5*, 65-72.
(6) Saleh, M. R. I.; Sabri, N. N.; El-Masry, S. *J. Pharm. Sci. U.A.R.* **1978**, *19*, 313-318.
(7) Saleh, M. R. I.; Sabri, N. N. *Egypt. J. Pharm. Sci.* **1979**, *20*, 411-415.
(8) Sabri, N. N.; Abou-Donia, A. A.; Assad, A. M.; Ghazy, N. M.; El-Lakany, A. M.; Tempesta, M. S.; Sanson, D. R. *Planta Medica*, in press.
(9) (a) Tempesta, M. S.; Sanson, D. R.; Schlemper, E. O.; Sabri, N. N.; Abou-Donia, A. A.; El-Lakany, A. E., presented in part in the 16th International Symposium on the Chemistry of Natural Products (IUPAC), May 29-June 3, 1988, Kyoto, Japan; Abstr. PA132. (b) Sanson, D. R.; Tempesta, M. S.; El-Lakany, A. E.; Abou-Donia, A. A., presented in part at the 22nd Midwest Regional Meeting of the American Chemical Society, Nov 5-6, 1987, Wichita, KS; Abstr. 616.
(10) Kakisawa, H.; Hayashi, T.; Okazaki, I.; Ohashi, M. *Tetrahedron Lett.* **1968**, *28*, 3231-3234.

0022-3263/89/1954-4097$01.50/0

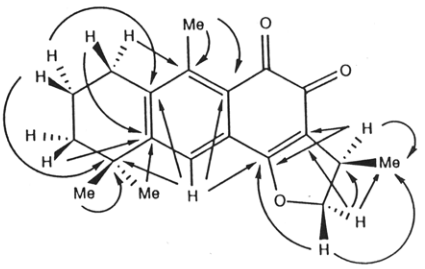
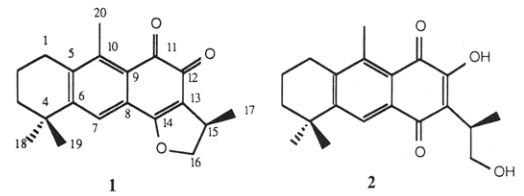


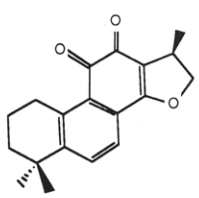
Figure 1. Summary of long-range heteronuclear couplings observed on aegyptinone A (1) using INAPT.

^{1}H NMR spectrum also showed ($CDCl_3$, Table I) a close resemblance in most of its features to those of cryptotanshinone (**3**).[3] However, two main differences were apparent in the spectrum of quinone **1**: (i) One benzenoid proton at δ 7.58 (s) in **1** replaced the AB quartet of the two benzenoid protons of cryptotanshinone (**3**) appearing at δ 7.15 and 7.30 (J_{AB} = 8 Hz),[3] (ii) the addition of a deshielded singlet at δ 2.60 (3 H) attributed to an aromatic methyl group located *peri* to a carbonyl group. These differences pointed to a rearranged structure. The dihydromethylfuran ring moiety was consistent with the following ^{1}H NMR data: a methyl doublet at δ 1.38 (C-17, 3 H, J = 6.8 Hz) coupled to a methine multiplet at δ 3.60 (C-15), 1 H), which was coupled to α and β methylene protons at δ 4.39 (C-16α, 1 H, dd, J = 8.7, 5.5 Hz) and δ 4.90 (C-16β, 1 H, t, J = 8.7 Hz). The ^{1}H NMR spectrum also revealed two benzylic protons at δ 2.75 (b t) ascribed to the C-1 methylene and a *gem*-dimethyl group at δ 1.35 (s, 6 H). NOESY and DNOES data indicated that the *gem*-dimethyls at δ 1.35 were close to the aromatic proton at δ 7.58, and that the C-1 methylene group at δ 2.75 was close to the aromatic methyl at δ 2.60. The above ^{1}H NMR data are suggestive of structure **1** as shown below. Normal, QUAT, and DEPT ^{13}C NMR spectra gave carbon chemical shifts and multiplicities (Table I) in accord with a substituted *o*-naphthoquinone. In particular, the ^{13}C NMR spectrum ($CDCl_3$) supported the presence of an orthoquinone system by the observation of two quaternary carbon signals at δ 184.5 and 176.2. Moreover, the signals at δ 171.0 (s) and 81.3 (t), assigned as C-14 and C-16, respectively, reflect their presence in an ether linkage involving sp^2 and sp^3 carbons. Significant heteronuclear long-range coupling correlations obtained from INAPT, as shown in Figure 1, allowed carbon assignments to be made, as well as completing of the structure of **1** as depicted. The conclusions drawn from spectroscopic analyses of **1** were corroborated by the molecular structure obtained by single-crystal X-ray diffraction techniques.

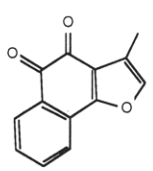


1

2



3



4

Aegyptinone B (**2**), $C_{20}H_{24}O_4$ (HRMS), was isolated as dark red plates, mp 101 °C. The UV and IR spectra were

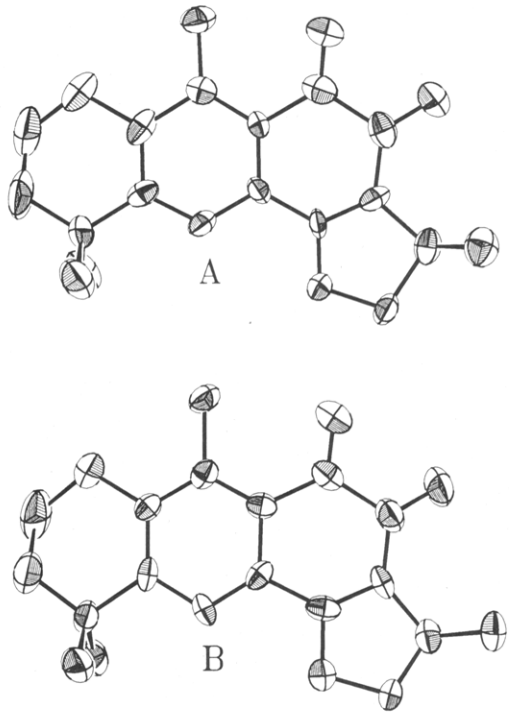


Figure 2. Computer-generated perspective drawing (ORTEP) of independent molecules A and B of aegyptinone A (**1**) with correct absolute stereochemistry shown and hydrogens ommitted for clarity.

similar to those of **1**. Additionally, both alcoholic and phenolic hydroxyls bands (3440 and 3340 cm^{-1}) were observed in the IR spectrum of **2**. The ^{13}C and ^{1}H NMR parameters of **2** given in Table I are also similar to those of compound **1**. Significant ^{1}H NMR differences were as follows: H-7 (δ 7.58, s in **1**, δ 8.12, s in **2**) is deshielded in **2** due to becoming *peri* to a carbonyl; H-16 methylene protons (δ 4.39, dd, and δ 4.90, t in **1**; δ 3.95 and 3.89, both multiplets in **2**) are shifted upfield in **2** consistent with ring opening of the dihydrofuran moiety in **1**. This postulate was secured by the MS spectrum of **2**, which exhibited fragments at m/z 298 (base peak) and 297 (58%) due to loss of CH_2OH from the molecular ion. The signal at δ 12.86 in the ^{1}H NMR confirmed the presence of a strongly chelated phenolic hydroxyl, consistent with the *p*-quinone as the major tautomer of **2** as shown. The most significant difference in the ^{13}C NMR spectra of the two quinones is the triplet at δ 81.3 for C-16 in **1** is shifted upfield to δ 66.0 in **2**, indicating the dihydrofuran ring in **1** has been opened as shown in **2**.

The structure of these two naturally occurring quinones is closely related to the synthetic *o*-naphthoquinone derivative **4**, which exhibited prominent cytotoxicity against KB cells.[11] Moreover, it was reported[12] that some of the major tanshinones of *Salvia militiorrhiza* showed significant activity against KB cells; and it was speculated that saturation in the ring A of these diterpenoids might be responsible for the antineoplastic activity. The unusual rearranged abietane skeleton of **1** and **2** has also been recently reported from another source (*Pygmaeopremna herbacea*).[13] Biosynthetically, the novel carbon skeleton of **1** and **2** may arise from 6,7-didehydroroyleanone (**5**)[8] (commonly found in *Salvia*) as shown in Figure 3. The

(11) Hayashi, T.; Smith, F. T.; Lee, K.-H. *J. Med. Chem.* **1987**, *30*, 2005–2008.
(12) Wu, W. L.; Chang, W. L.; Lee, A. R.; Lin., H. C.; King, M. L. *J. Med. Sci.* **1985**, *6*, 159.
(13) Meng, Q.; Zhu, N.; Chen, W. *Phytochemistry* **1988**, *27*, 1151–1152.

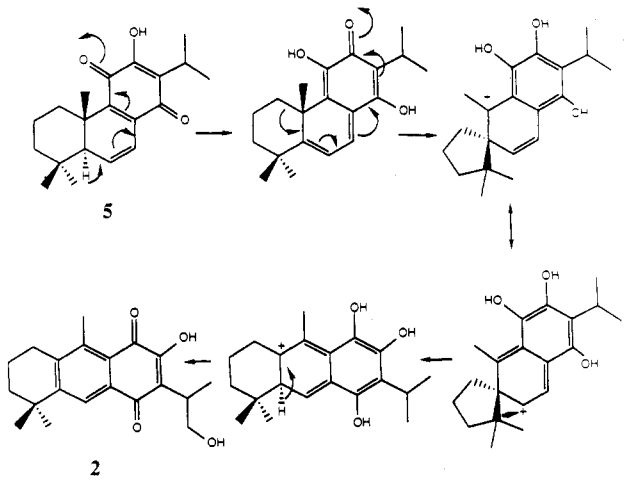


Figure 3. Proposed biosynthetic pathway from 6,7-dehydroroyleanone (**5**) to aegyptinone B (**2**).

numbering system shown in **1** follows that found in the abietanes. The absolute configuration shown for **1** and **2** at C-15 (*R*) is assumed to be the same as that determined for the structurally similar cryptotanshinone (**3**).[17]

Experimental Section

Physical Analyses. Melting points were determined on a Koffler hot stage microscope and are uncorrected. IR and UV spectra were recorded on a Beckman Model 4210 and Pye Unicam Sp 8-100 UV/VIS spectrophotometers, respectively. MS of the samples were obtained on a Kratos MS-25 mass spectrometer at 70 eV and 1 s/decade for low resolution, and at 70 eV and 3 s/decade for measurements, of exact masses. NMR experiments were performed on a Nicolet NT-300 WB spectrometer equipped with 5-mm ^1H and ^{13}C NMR single-frequency probes operating at 300 and 75.5 MHz, respectively. All ^1H NMR chemical shifts were referenced to internal TMS (0.00 ppm). The homonuclear 2D COSY and NOESY experiments used a delay of 3 s and a 90-deg pulse of 8 μs. The pulse sequences BILEV and DEPT were used to obtain ^{13}C NMR spectra. The heteronuclear spin-echo pulse sequence QUAT[14] generated a ^{13}C subspectrum of only quaternary carbons, which otherwise were difficult to observe in **1**. We were unable to use QUAT to determine the quaternary carbons in **2** due to limited sample size and tautomerization. QUAT used a 90-deg pulse of 10 μs with a 12-s delay and $\tau = {}^1/_2J$ (3.1 ms) optimized for a J value of 160 Hz. The proton 90-deg decoupler pulse has been calibrated to 33 μs with a power setting similar to that used in DEPT. The sensitive 1D long-range heteronuclear chemical shift correlation experiment INAPT[15,16] was performed on **1** with a 90-deg carbon pulse of 11 μs and a 90-deg proton soft pulse of 10 ms. The experiment was optimized to observe coupling of 8–14 Hz.

Plant Material. Samples were collected from wild plants growing on the Mediterranean coastal strip 60 km west of Alexandria, Egypt. The plants were identified by Professor Dr. L. Boulos of the National Research Centre, Cairo, Egypt. Voucher specimens are on deposit in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University.

Extraction and Isolation. The air-dried powdered roots (1 kg) were extracted with petrol in a Soxhlet apparatus. The residue (9 g) after removal of the solvent was chromatographed over a column of silica gel (270 g, Merck, 70–230 mesh). Elution was performed with petroleum ether $CHCl_3$ solvent mixtures; 60 fractions, 200 mL each, were collected. Preparative TLC of each of fractions (20–26) and (27–29) using petroleum ether–EtOAc, 8:2, afforded successively two minor quinones. Each of fractions (40–43) and (52–60) contained one major colored spot. Repeated crystallization of each of these fractions from MeOH gave compounds **1** and **2**, respectively.

Aegyptinone A (1): dark orange prisms; mp 135–136 °C (MeOH); yield 0.075%; UV λ_{max} (MeOH) 212, 222, 268, 277, 296, 360, and 477 nm; IR (film) 3020, 2950, 2920, 2860, 1680, 1660, 1640, 1610, 1570, 1553, 1540, 1460, 1450, 1405, 1390, 1370, 1345, 1280, 1260, 1210, 1185, 1160, 1165, 1050, 995, 920, 900, 885, 810, 790 cm^{-1}; ^1H and ^{13}C NMR, results are summarized in Table I; HRMS m/z (rel int) found $[M]^+$ 310.3946, calcd for $C_{20}H_{22}O_3$ $[M]^+$ 310.3940; 312 $[M^+ + 2]$ (1.5), 311 $[M^+ + 1]$ (7), 310 (25) $[M]^+$, 295 (4.5), 283 (13), 282 (61), 268 (69), 267 (100), 253 (4), 239 (4), 237 (4), 223 (6), 209 (3), 195 (3), 186 (4), 185 (25), 178 (4.5), 165 (9), 152 (5.5), 142 (9), 141 (10), 129 (5), 128 (7), 115 (7.5), 109 (3), 103 (1.5), 99 (1), 91 (3), 89 (3), 83 (2), 77 (4), 69 (1), 65 (2), 63 (2), 55 (2), 53 (3).

Aegyptinone B (2): Dark red plates; mp 101–102 °C (MeOH); yield 0.015%; UV λ_{max} (MeOH) 229, 280, 345, and 460 nm; IR (film) 3440, 3330, 3030, 2950, 2920, 2845, 1675, 1655, 1632, 1612, 1575, 1555, 1455, 1540, 1410, 1370, 1345, 1280, 1230, 1120, 1030, 750 cm^{-1}; ^1H and ^{13}C NMR data are listed in Table I; HRMS m/z (rel int) found $[M]^+$ 328.1688, calcd for $C_{20}H_{24}O_4$ 328.1674; 330 $[M^+ + 2]$ (3), 329 $[M^+ + 1]$ (18), 328 $[M]^+$ (72), 313 (14), 310 (23), 299 (28), 298 (100), 297 (58), 283 (26), 282 (18), 267 (37), 253 (17), 239 (18), 226 (10), 224 (8), 201 (10), 185 (23), 178 (10), 165 (18), 152 (17), 141 (22), 128 (23), 115 (20), 95 (10), 91 (10), 77 (14), 69 (20), 55 (20), 43 (28), 41 (38), 39 (29), 31 (50).

Single-Crystal X-ray Analysis of Aegyptinone A (1). Crystal data for quinone **1**, $C_{20}H_{22}O_3$: dark orange prismatic crystal, 0.2 × 0.3 × 0.35 mm, monoclinic, space group $P2_1$, a = 11.295 (3) Å, b = 11.653 (4) Å, c = 12.771 (6) Å, β = 100.8 (1)°, V = 1651 (2) Å^3, and ρ_{calc} = 1.249 g/cm^3 for Z = 4. Diffraction data: Enraf-Nonius CAD4 automated k-axis diffractometer, graphite-monochromated Mo radiation ($\lambda(K\alpha)$ = 0.71069 Å), range $2.0 < 2\theta < 50.0°$, 3985 reflections (3673 unique, R_i = 0.013, 1963 observed, $I > 4.0\sigma(F)$); corrected for anomalous dispersion, Lorentz, and polarization effects. Solution: direct methods (SHELXS-86)[18] and difference Fourier syntheses, hydrogen atoms included in calculated positions riding on attached carbon atoms owing to paucity of data. Refinement: anisotropic thermal coefficients for non-hydrogen atoms, isotropic group thermal parameter for hydrogen atoms (SHELX-76).[19] Final: no significant features in the difference Fourier map ((range $-0.30 <$ e/Å$^3 < +0.30$); agreement factors, R = 0.055, R_w = 0.065, and S = 2.44. Minor disorder about C-1, C-2, and C-3 of the saturated ring in both crystallographically independent molecules is indicated by high thermal parameters and deviation from ideal geometry (Figure 2).

Acknowledgment. We are grateful to Prof. R. Tadross, Department of Microbiology, Faculty of Pharmacy, Alexandria University, for the antimicrobial screening and to M. A. Abou-Karam, University of Minnesota, for running the HRMS of quinones **1** and **2**. We are also grateful for financial support (in part) to the National Science Foundation for the NMR (PCM-8115599) and MS (PCM-8117116) Facilities, and to the University of Missouri Institutional Biomedical Research Support Grant (PR07053) from the National Institutes of Health.

Registry No. **1**, 121704-42-5; **2**, 121704-43-6.

Supplementary Material Available: Tables of fractional coordinates, bond distances, bond angles, and anisotropic thermal parameters (5 pages); table of observed/calculated structure factors for aegyptinone A (**1**) (7 pages). Ordering information is given on any current masthead page.

(14) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Johns, S. R.; Willing, R. I. *J. Chem. Soc., Chem. Commun.* **1982**, 1138.
(15) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314.
(16) Taylor, R. B.; Corley, D. G.; Tempesta, M. S.; Fomum, Z. T.; Ayafor, J. F.; Wandji, J. *J. Nat. Prod.* **1986**, *49*, 670–673.
(17) Tomita, Y.; Ikeshiro, Y. *J. Chem. Soc., Chem. Commun.* **1987**, 1311–1313.
(18) Sheldrick, G. M. *SHELX 76*: Program *for Crystallographic Structure Determination*; University of Cambridge, UK; 1976.
(19) Sheldick, G. M. In *Crystallographic Computing 3*; Sheldrick, G. M., Kruger, C., Godard, R., Eds.; Oxford University Press: London, 1985; pp 175–189.