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

Two new diterpene quinones from the roots of *Salvia lanigera* Poir.

A. M. El-Lakany

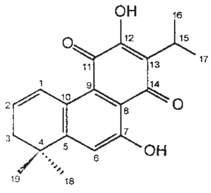
Received July 9, 2002
Accepted July 12, 2002

Ass. Prof. Dr. Abdalla El-Lakany, University of Alexandria, Faculty of Pharmacy, Department of Pharmacognosy, Alexandria, Egypt

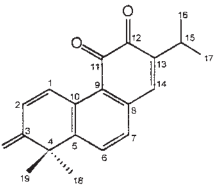
Pharmazie 58: 75–76 (2003)

Plants of the genus *Salvia* are commonly used in folk-medicine for a wide variety of remedies [1] and are of commercial interest in the production of essential oils and flavoring agents [2]. Members of the genus *Salvia* have yielded a diversity of secondary metabolites including flavonoids, triterpenoids and diterpenoids of neo-clerodane and abietane types. In addition, a great number of diterpene quinones have been isolated. It is to be mentioned that the presence of diterpene quinones can be used as a chemotaxonomical marker of the genus [3, 4]. Previous studies on the roots of *Salvia lanigera* growing in Egypt resulted in the isolation and identification of several diterpenoids [5–10]. Here we report the isolation and identification of two new abietane diterpene quinones namely sanigerone (**1**) and saligerone (**2**) from the roots of *Salvia lanigera* growing in Egypt.

The finely powdered roots of *S. lanigera* were extracted with chloroform. The dry chloroformic extract on subjection to silica gel column chromatography and preparative TLC furnished sanigerone (**1**) and saligerone (**2**).



Sanigerone (1)



Saligerone (2)

Sanigerone (**1**) was isolated in the form of a reddish gum. The elemental formula of sanigerone was established to be $C_{19}H_{20}O_4$ from EIMS that revealed the presence of the molecular ion peak at m/z 312 and ^1H NMR spectrum, integrated for 20 protons. In addition, ^{13}C NMR spectrum revealed the presence of 17 resolved signals representing 19 carbons. DEPT experiment indicated these carbons to be: 4 CH_3, CH_2, 4 CH and 10 quaternary carbons of which four are oxygenated (2 quinonoidal and 2 aromatic carbons). ^1H NMR spectrum showed the presence of three signals at δ 1.28 (s, 6 H), at δ 1.30 (d, J = 7.1 Hz, 6 H) and at δ 3.37 (hept, J = 7.1 Hz, 1 H) due to gem dimethyl and isopropyl groups. HMQC indicated their corresponding carbons to be at δ 28.38, at δ 19.75 and at δ 23.87 respectively. This indicated that sanigerone (**1**) has a normal abietane skeleton but lacking the angular methyl at C-10. The presence of two hydroxyl groups *peri* to the two quinonoidal carbonyls at positions 7 and 12 was established, as the IR spectrum showed two strong bands at 3479 and 3330 cm^{-1}, while the ^1H NMR spectrum revealed the presence of two singlets exchangeable with D_2O; at δ 13.60 and at δ 7.90. HMBC experiments indicated their corresponding carbons to be at δ 153.75 and at δ 162.14, respectively. The IR spectrum showed also three bands at 1662, 1645 and 1616 cm^{-1} due to chelated quinonoidal carbonyls. An ABX system was observed in the ^1H NMR spectrum, at δ 8.00 (dt, J = 10.0, 1.8 Hz, 1 H), δ 6.31 (dt, J = 10.0, 4.7 Hz, 1 H) and δ 2.27 (dd, J = 1.8, 4.7 Hz, 2 H) due to protons at positions 1, 2 and 3, respectively. HMQC showed their corresponding carbons to be at δ 124.51 (d), at δ 133.73 (d) and at δ 37.65 (t) respectively. In the ^1H NMR spectrum, the singlet at δ 7.24 was assigned for proton at position 6 as HMBC experiment indicated this singlet to have a relation with carbons at δ 34.86, δ 112.76, δ 162.14 and δ 129.63. Full assignment of the chemical structure of sanigerone (**1**) was made possible through extensive study of 2D-NMR spectra (^1H, ^1H-COSY, HMQC and HMBC).

Saligerone (**2**) was isolated in the form of dark orange gum. UV and IR absorption spectra were consistent with an ortho-quinone chromophore [11]. The *o*-quinone moiety was also supported by MS that exhibited an M^{+2} peak more intense than the molecular ion peak [12, 13]. The ^1H NMR spectrum; integrated for 18 protons; which when coupled with ^{13}C NMR spectra (normal and DEPT) and the observed molecular ion in the EI-MS, suggested the molecular formula of **2** as $C_{19}H_{18}O_3$. As in **1**, the ^1H NMR spectrum of **2** showed the presence of isopropyl and gem dimethyl groups, indicating that **2** incorporates normal abietane skeleton but lacking the angular methyl at C-10. The ^{13}C NMR spectrum of saligerone (**2**) revealed the presence of 17 resolved signals representing 19 carbons similar to **1**. DEPT experiments showed these carbons to be: 4 CH_3, 6 CH and 9 quaternary carbons of which three are oxygenated appearing at δ 181.20, 183.10 (quinonoidal) and 202.12. The ^1H NMR spectrum showed the presence of one singlet at δ 7.13 due to H-14 and two doublets (J = 7.9 Hz) at δ 7.66 and 7.35 due to H-6 and H-7 respectively. It also confirmed that **2** contains an α, β-unsaturated carbonyl moiety as two doublets (J = 10.7 Hz) were obtained at δ 8.98 and 6.40 due to H-1 and H-2 respectively. 2D-NMR spectra (^1H, ^1H-COSY, HMQC and HMBC) allowed full assignment and confirmed the structure of **2**.

Sanigerone was tested for its antimicrobial activity following the method suggested by Egorov [14]. It was found to be active against *Staph. aureus* (MIC = 13 μg/ml), *Ps. aeruginosa* (MIC = 13 μg/ml) and *C. albicans* (MIC = 52 μg/ml).

Experimental

1. General procedures

UV spectra were determined on Perkin Elmer double beam spectrophotometer Model 550 S, attached to a Hitachi recorder Model 561, with a fixed slit width of 2 nm and using 1 cm quartz cell. IR spectra were recorded on Beckmann model 4210 Infrared spectrophotometer in KBr pellet. EI-MS was carried out using Finnigan mass spectrometer Model SS Q/7000 (70 ev). ^1H NMR, ^{13}C NMR (normal, DEPT) and 2D-NMR spectra were recorded on Varian spectrometer 400 MHz for ^1H NMR and 90 MHz for ^{13}C NMR.

2. Plant material

Salvia lanigera Poir. was collected during March to April 1991 and 1992 from wild strands growing on the Mediterranean coastal strip 60 km west of Alexandria, Egypt. The plant was identified by comparison with a voucher sample (NA9) deposited in the Department of Pharmacognosy, Alexandria University, Alexandria, Egypt.

3. *Extraction, isolation and purification of the compounds*

Dry powdered roots (12 kg) were exhaustively extracted in soxhlet apparatus with $CHCl_3$. The dry extract (180 g) was fractionated over 360 g of silica gel with gradient light petroleum: $CHCl_3$ to give four fractions [14]. All fractions are kept in the refrigerator. The first fraction (50 g, light petroleum/$CHCl_3$, 1 : 1) was fractionated over 800 g of silica gel, and 15 fractions were obtained.

3.1. Sanigerone (7,12-dihydroxy-1,5(10),6,8,12-abietapentaene-11,14-dione) (***1***)

Fraction 3 (7.5% chloroform in petroleum ether) was subjected to column and preparative TLC (petroleum ether: ethyl acetate; 49 : 1) to give 60 mg of sanigerone (**1**). UV (λ_{max}, MeOH) 297, 427, 487 nm. IR (KBr, ν, cm^{-1}) 3479, 3330, 2963, 2928, 1662, 1645, 1612, 1449, 1439, 1396, 1372. EIMS m/z (rel. int.) 312 (100), 297 (87), 283 (43), 279 (75), 269 (77), 251 (52), 237 (14), 201 (32), 165 (35), 128 (29), 115 (16), 83 (16), 69 (13), 55 (10). ^{1}H NMR ($CDCl_3$) δ 8.00 (1 H, dt, J = 10.0, 1.8 Hz, H-1), 6.31 (1 H, dt, J = 10.0, 4.7 Hz, H-2), 2.27 (2 H, dd, J = 1.8, 4.7 Hz, CH_2-3), 7.24 (1 H, s, H-6), 13.60 (1 H, s, $O\underline{H}$-7), 7.90 (1 H, s, $O\underline{H}$-12), 3.37 (1 H, hept., J = 7.1 Hz, H-15), 1.30 (6 H, d, J = 7.1 Hz, $C\underline{H}_3$-16 and $C\underline{H}_3$-17), 1.28 (6 H, s, $C\underline{H}_3$-18 and $C\underline{H}_3$-19). ^{13}C NMR ($CDCl_3$) δ 124.51 (C-1), 133.73 (C-2), 37.65 (C-3), 34.86 (C-4), 155.36 (C-5), 122.62 (C-6), 162.14 (C-7), 112.76 (C-8), 121.98 (C-9), 129.63 (C-10), 183.05 (C-11), 153.75 (C-12), 126.56 (C-13), 190.57 (C-14), 23.87 (C-15), 19.75 (C-16 and C-17), 28.38 (C-18 and C-19).

3.2. Saligerone (1,5(10),6,8,13-abietapentaene-3,11,12-trione) (**2**)

Fraction 10 (45% chloroform in petroleum ether) was subjected to repeated column and preparative TLC (petroleum ether: ethyl acetate; 45 : 5) to give 25 mg of saligerone (**2**), UV (λ_{max}, MeOH) 269, 324, 381 nm. IR (KBr, ν, cm^{-1}) 3058: 2925, 1713, 1695, 1663, 1646, 1629, 1571, 1467, 1450, 1388. EIMS m/z (rel. int.) 296 [M^{+2}] (14), 294 (12), 266 (29), 251 (12), 238 (21), 223 (35), 195 (13), 43 (100). ^{1}H NMR ($CDCl_3$) δ 8.98 (1 H, d, J = 10.7 Hz, H-1), 6.40 (1 H, d, J = 10.7 Hz, H-2), 7.66 (1 H, d, J = 9.7 Hz, H-6), 7.35 (1 H, d, J = 7.9 Hz, H-3), 7.13 (1 H, s, H-14), 3.04 (1 H, hept., J = 6.9 Hz, H-15), 1.18 (6 H, d, J = 6.9 Hz, $C\underline{H}_3$-16 and $C\underline{H}_3$-17), 1.48 (6 H, s, $C\underline{H}_3$-18 and $C\underline{H}_3$-19). ^{13}C NMR ($CDCl_3$) δ 139.60 (C-1), 128.85 (C-2), 202.12 (C-3), 47.88 (C-4), 150.40 (C-5), 132.58 (C-6), 131.17 (C-7), 135.47 (C-8), 127.07 (C-9), 132.23 (C-10), 183.10 (C-11), 181.20 (C-12), 146 (C-13), 139.12 (C-14), 27.19 (C-15), 21.53 (C-16 and C-17), 27.61 (C-18 and C-19).

Acknowledgement: The author thanks Dr. Ihab Essa, Lecturer at the Department of Microbiology (Diary Products), Faculty of Agriculture, University of Alexandria, for measuring the antimicrobial activity of sanigerone.

References

1 Duke, J. A.: Handbook of Medicinal Plants, p. 419, CRC Press, Inc. Boca Raton, F. L., 118, 1985
2 Lawrence, B. M.; in: Harley, R. M.; Reynolds, T. (ed.): Advances in Labiatae Science, p. 399, The Royal Botanic Gardens, Kew, U. K., 1992
3 Romanova, A. S.; Patudin, A. V.; Vlasova, G. F.; Bankovskii, A. I.: Rast. Resur. **9** (2), 218 (1973)
4 Patudin, A. V.; Romanova, A. S.; Sokolow, W. S.; Pribylova, G.: Planta Med. **26**, 201 (1974)
5 Saleh, M. R.; Sabri, N. N.; El-Masry, S.: Egypt. J. Pharm. Sci. **19**, 313 (1978)
6 Sabri, N. N.; Abou-Donia, A. A.; Assad, A. M.; Ghazy, N. M.; El-Lakany, A. M.; Tempest, M. S.; Sanson, D. R.: Planta Med. **55**, 5821 (1989)
7 El-Lakany, A. M.; Sabri, N. N.: Alex. J. Pharm. Sci. **6**, 159 (1992)
8 El-Lakany, A. M.; Abdel-Kader, M. S.; Sabri, N. N.; Stremitz, F. R.: Planta Med. **61**, 559 (1995)
9 Lee, I. S.; Kaneda, N.; Suttisri, R.; El-Lakany, A. M.; Sabri, N. N.; Kinghorn, A. D.: Planta Med. **64**, 632 (1998)
10 Aboul-Ela, M. A.; El-Lakany, A. M.: Alex. J. Pharm. Sci. **14** (1), 57 (2000)
11 Kakisawa, H.; Hayashi, T.; Okazaki, I.: Tetrahedron Lett. **28**, 3231 (1968)
12 Ukai, S.; Hirose, K.; Tatematsu, A.; Goto, T.: Tetrahedron Lett. 4999 (1967)
13 Olivar, R. W. A.; Rashman, R. M.: J. Chem. Soc. (B), 1141 (1968)
14 Egorov, N. S.; Loriia, Zh. K.; Vybornykh, S. N.; Khamrun, R.: Nauchnye. Doki. Vyss. Shkoly. Biol. Nauki. **6**, 89 (1985)

G. K. Skjabin-Institute of Biochemistry and Physiology of Microorganisms[1], Russian Academy of Sciences, Pushchino na Oke, Moscow Region, Russia, Hans-Knöll-Institute for Natural Products Research[2], Jena, Germany

Nosporins A and B, new metabolites from a filamentous fungus, VKM-3750

A. G. Kozlovsky[1], V. P. Zhelifonova[1], V. M. Adanin[1],
S. M. Ozerskaya[1], U. Gräfe[2]

Received March 5, 2001
Accepted December 12, 2001

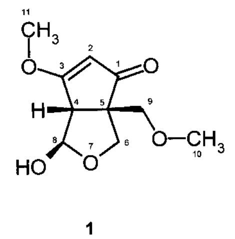
Prof. Dr. U. Gräfe, Hans-Knöll-Institut für
Naturstoff-Forschung e. V.,
Beutenbergstr. 11a, D-07745 Jena, Germany
ugraefe@pmail.hki-jena.de

Pharmazie 58: 76–77 (2003)

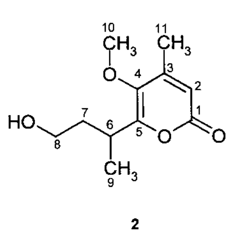
In the course of our screening for new metabolites from fungi [1] we investigated culture extracts of the taxonomically identified fungus VKM F-3750 using mass spectrometry and staining behaviour on TLC as a screening feature. This fungal strain was isolated from a forestal region near Pushchino (Moscow region, Russia). It was deposited as strain VKM-3750 in the All Russian Culture Collection (VKM) of the G. K. Skrjabin-Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino near Moscow, Russia).

Colonies of the fungal strain VKM-3750 on potato dextrose agar (PDA) and malt-extract agar (MA) are growing rapidly attaining a diameter of 35–45 mm in subcentral areas, azonate or indistinctly zonate near colony margins. Vegetative hyphae are white to shightly brownish, passing towards greyish-brownish with aging. Often the hyphae display bulbous and other nonconidial structures. Conidiogenes were absent. The strain reversed usually in drab to brownish or blackish shades. Formation of spores was not observed as this was observed with representatives of the agromomycetes, hyphomycetes or mitosporic fungi.

Cultivation occurred for 120 h under submerged conditions in 750 ml Erlenmeyer flasks containing 150 ml of a medium composed of (g/L): mannitol 20, succinic acid 5.4, $MgSO_4 \cdot 7\,H_2O$ 0.3 and KH_2PO_4 1 (24 °C, rotary shakers 220–240 r.p.m.). The culture broth was adjusted to pH 8.0–8.5 by NH_4OH and extracted twice by $CHCl_3$ in ratio 1 : 1. The residue of the evaporated culture extract was fractionated using several subsequent chromatographic steps such as column chromatography on silica gel and preparative thin-layer chromatography on normal and reverse phase silica gel. Finally 15.8 mg of metabolite A (**1**) and 13.0 mg of B (**2**) were isolated as waxy mass from 3.5 L culture broth. **1** and **2** were given the name



1



2