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DITERPENES AND STEROLS FROM THE ROOTS OF *Salvia verbenaca* subsp. *clandestina*

A. Kabouche,[1*] Z. Kabouche,[1]
R. Touzani,[2] and C. Bruneau[3]

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The genus *Salvia* (Lamiaceae) comprises some 250 species widely distributed in Turkey, Mexico, and China. Twenty four species and subspecies grow in Algeria [1]. This genus is found to be rich with diterpenoids, which can be divided into two categories. The first one comprises one monocyclic, 2,6-dimethyl-10-(*p*-tolyl)undeca-2,6-(*E*)-diene, isolated from *S. dorisiana* [2], and bicyclic diterpenoids including labdanes, clerodanes, and related diterpenoids. The second category contains tricyclic and tetracyclic diterpenoids including pimaranes, abietanes, and related rearranged diterpenoids. The clerodane-type diterpenoids characterize the American species, while the abietane type are represented by the European, Asian, and North African species. We have found more than 545 diterpenoids isolated from *Salvia* species. As part of our study of the genus *Salvia* [3, 4] and the Lamiaceae in general [5–8], we report here the phytochemical study of the roots of *Salvia verbenaca* subsp. *clandestina*, which has not been the subject of any study, but flavonoids [9], abietane-type diterpenoids, namely taxodione, horminone, 6β-hydroxy-7α-acetoxyroyleanone [10] verbenacine, and salvinine [11] were reported from the Egyptian species *S. verbenaca*.

Salvia verbenaca subsp. *clandestina*, an endemic species of the genus *Salvia*, was collected from Batna (Eastern Algeria). The plant material was authenticated by Prof. Gerard De Belair (University of Annaba, Algeria) and a voucher specimen (LOST/ZKAK Svj05/00) was deposited at the Herbarium of the Faculty of Sciences, University Mentouri-Constantine and at the Herbarium of the Museum National d'Histoire Naturelle (MNHN) de Paris, France (P 00278250).

Air-dried and powdered roots (500 g) of *S. verbenaca* subsp. *clandestina* were extracted in a Soxhlet apparatus with Me_2CO to yield, after dryness, 5.6 g dry extract. This was subjected to CC on silica gel with a gradient of cyclohexane–EtOAc of increasing polarity. Compound **1** (5 mg) and compound **2** (3 mg) were isolated at a low polarity (99 : 1). The collected fractions, eluted with cyclohexane–EtOAc (97 : 3), were purified by preparative TLC on silica gel, eluted with *n*-hexane-dichoromethane (80 : 20), leading to compound **3** (5 mg). Compounds **4**–**6** were found in the fraction eluted with cyclohexane–EtOAc (85:15). The collected fractions, eluted with cyclohexane–EtOAc (75:25), were subjected to a CC on silica gel eluted with mixtures of cyclohexane–EtOAc with increasing polarity, affording compound **7** (2 mg).

Compound **1,** $C_{20}H_{26}O_3$, *m/z*: 332 $[MNH_4]^+$, IR spectrum (ν_{max}, cm^{-1}): 1638, 1699 (ortho or paraquinone), 3435 (OH); UV spectrum (MeOH, λ_{max}, nm): 261 (2.83), 330 (2.54), 461 (0.25).

^{1}H NMR (250 MHz, δ, ppm, J/Hz): 2.9 (1H, m, H-1β), 2.1 (1H, t, $J_{AX} + J_{BX} = 6$, H-5), 6.5 (1H, dd, J = 3; 12, H-6), 6.8 (1H, dd, J = 3.0; 12.0, H-7), 7.3 (1H, s, H-12), 3.2 (1H, sept, J = 7, H-15), 1.2 (1H, d, J = 7, H-16), 1.2 (1H, d, J = 7, H-17), 0.9 (1H, s, H-18), 1.0 (1H, s, H-19).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 35.2 (C-1), 18.7 (C-2), 41.6 (C-3), 40.6 (C-4), 52.2 (C-5), 139.6 (C-6), 121.1 (C-7), C-9 (138.5), C-8 (140.6), C-10 (33.8), C-14 (183.5), C-11 (185), C-12 (151.6), C-13 (122.6), C-15 (22.8), C-17 (19.8), C-16 (20.0), C-18 (24.1), C-19 (33.3), C-20 (15.2). Identified as 6,7-dehydroroyleanone [12].

Compound **2,** $C_{20}H_{28}O_3$, *m/z*: 334 $[MNH_4]^+$, IR spectrum (ν_{max}, cm^{-1}): 3475, 3362 (OH), 2962, 1552, 1506 (double bond), 1625 (carbonyl); UV spectrum (MeOH, λ_{max}, nm): 261.5 (3.32), 333.8 (2.06) (double bond and an aromatic ring).

^{1}H NMR (250 MHz, δ, ppm, J/Hz): the same as for compound **1** except with the appearance of two singlets at 7.5 and 13.0 ppm attributed to OH-11 and OH-14, respectively.

1) Laboratoire d'Obtention de Substances Therapeutiques (L.O.S.T), Faculte des sciences, Universite Mentouri - Constantine, Campus Chaabat Ersas, 25000 Constantine, Algeria, fax: 213 31 81 88 59, e-mail: ahkabouche@yahoo.fr; 2) Universite Mohammed Premier, Faculte Pluridisciplinaire, Nador, Marocco; 3) Universite de Rennes 1, Campus de Beaulieu, 35042 Rennes cedex, France. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 663-664, November-December, 2008. Original article submitted May 1, 2007.

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^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): the same as for compound **1** except with the appearance of two carbonyl signals at 183 and 185 ppm attributed to C-11 and C-14, respectively. Identified as 11,12,14-trihydroxyabieta-6,8,11,13-tetraene or cryptanol [13].

Compound **3,** $C_{20}H_{26}O_2$, *m/z*: 298 $[M]^+$, IR spectrum (ν_{max}, cm^{-1}): 1651 (carbonyl), 3425 (OH), 2964, 1595, 1562 (aromatic ring); UV spectrum (MeOH, λ_{max}, nm): 261.5 (3.32), 333.8 (2.06) (double bond and an aromatic ring).

^{1}H NMR (360 MHz, δ, ppm, J/Hz): 3.61 (1H, ddd, J = 14; 12; 2, H-1β), 2.79 (1H, ddd, J = 14; 7; 2, H-1α), 1.81 (1H, m, H-2α), 1.43 (1H, m, H-2β), 2.37 (1H, m, H-3α), 1.28 (1H, m, H-3β), 7.06 (1H, d, J = 7, H-6), 6.90 (1H, d, J = 7, H-7), 4.51 (1H, s, H_{11}-OH), 6.97 (1H,d, J = 1, H-14), 3.02 (1H, d sept, J = 6; 1, H-15), 1.22 (3H, d, J = 7, H-16), 1.17 (3H, d, J = 7, H-17), 0.79 (3H, s, H-18), 0.81 (3H, s, H-19), 2.37 (3H, s, H-20).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 26.9 (C-1), 23.5 (C-2), 42.9 (C-3), 39.0 (C-3), 137.4 (C-5), 130.1 (C-6), 126.7 (C-7), 129.1 (C-8), 139.4 (C-9), 143.3 (C-10), 84.4 (C-11), 206.1 (C-12), 141.0 (C-13), 141.1 (C-14), 27.1 (C-16), 22.7 (C-17), 22.1 (C-18), 28.0 (C-19), 21.4 (C-20). Identified as microstegiol [14].

Compound **4,** $C_{29}H_{48}O$, GC/MS with Wiley literature confirmed the structure of stigmasterol [15].

Compound **5,** $C_{29}H_{50}O$, GC/MS with Wiley literature confirmed the structure of sitosterol [15].

Compound **6,** GC/MS with Wiley literature confirmed the structure of campesterol [15].

Compound **7,** $C_{20}H_{26}O_4$, *m/z*: 330 $[M]^+$, IR spectrum (ν_{max}, cm^{-1}): 2962, 1635, 1583, 1554 3522, 3383, 3240, 2872 (carbonyl and an aromatic ring); UV spectrum (MeOH, λ_{max}, nm): 250.20 (3.22), 287.4 (2.71), 341.2 (2.65).

^{1}H NMR (360 MHz, δ, ppm, J/Hz): 2.96 (1H, ddd, J = 14; 6; 2, H-1β), 7.74 (1H, s, H-14), 3.06 (1H, sept, J = 7, H-15), 1.30 (3H, d, J = 7, H-16), 1.32 (3H, d, J = 7, H-17), 1.46 (3H, s, H-18), 1.47 (3H, s, H-19), 1.68 (3H, s, H-20).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ, ppm): 30.5 (C-1), 17.9 (C-2), 36.5(C-3), 36.5 (C-4), 143.1 (C-5), 143.0 (C-6), 179.9 (C-7), 121.1 (C-8), 138.2 (C-9), 40.7 (C-10), 140.8 (C-11), 145.3 (C-12), 132.8 (C-13), 116.6 (C-14), 27.5 (C-15), 22.5 (C-16), 22.4 (C-17), 27.2 (C-18), 28.0 (C-19), 28.0 (C-20). Identified as 6-hydroxysalvonolone [16].

All the compounds are reported for the first time from the species *S. verbenaca* and the subspecies *clandestina.*

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