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Triterpenoids of the roots of *Lavandula stoechas* ssp. *stoechas*

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The roots of *Lavandula stoechas* ssp. *stoechas* afforded eleven known triterpenes, two steroids and two aromatics, in addition to two new triterpenes, 18-hydroxy-27-norolean-12,14-dien-30-al-28-oic acid and 3β-hydroxy-1-oxo-olean-12-ene-30-al-28-oic acid. Their structures were determined by spectroscopic analyses. The chloroform extract and some isolated compounds were evaluated for their growth inhibitory activity against several mammalian cell lines.

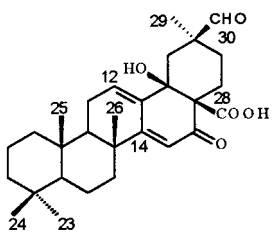
1. Introduction

Lavandula stoechas ssp. *stoechas* is an indigenous plant of Western Anatolia. In Turkey, there are two growing *Lavandula* subspecies of *Lavandula stoechas, ssp. stoechas* and *ssp. cariensis* besides the cultured *Lavandula* species, *L. angustifolia* [1]. *Lavandula* species have been used as expectorant, antispasmodic, carminative, stimulant, diuretic, and wound healing agents [2, 3]. There are some studies on the non-volatile constituents of *Lavandula* species [4–6]. In Turkey, previous studies on the aerial parts of *L. stoechas* ssp. *stoechas* led to the isolation of two longipinene derivatives [7] and some known triterpenes [8].

2. Investigations, results and discussion

In the present study, from the chloroform extract of the roots of *L. stoechas* ssp. *stoechas,* two new triterpenes, 18-hydroxy-27-norolean-12,14-dien-30-al-28-oic acid (**1**) and 3β-hydroxy-1-oxo-olean-12-ene-30-al-28-oic acid (**2**) were obtained. In addition, eleven known triterpenes α-amyrin (**3**) [9], oleanolic acid 3β-acetate (**4**) [10], betulin (**5**), betulinic acid (**6**) [11], 16β-hydroxylupeol-3-*O*-palmitate (**7**), 16β-hydroxylupeol-3-*O*-myristate (**8**) [12], 11-oxo-β-amyrin (**9**) [13], β-amyrin acetate (**10**), monogynol A *cis*-coumaryl ester (**11**), monogynol A *trans* coumaryl ester (**12**) [14], 3β,24-dihydroxyolean-12-ene (**13**) [15], two steroids, β-sitosterol (**14**)and β-sitosterol acetate (**15**), and two aromatics, *cis*-4-*O*-methyl caffeic acid octanol ester (**16**), *trans*-4-*O*-methyl caffeic acid octanol ester (**17**) [16] were isolated. Among these compounds, **7**, **8**, **9**, **11**, **12**, **16** and **17** were isolated for the first time from a *Lavandula* species.

For compound **1**, the molecular formula $C_{29}H_{40}O_5$ derived from its HRMS (m/z 468.2890) indicated ten degrees of unsaturation, five of which were accounted for by a pentacyclic ring system, and two by double bonds, and three by carbonyl groups. In the IR spectrum, two strong absorption bands were observed at 1720 and 1685 cm^{-1} with a shoulder at 2500 cm^{-1} indicating an aldehyde and acid moieties, respectively. There were also bands at 1660, 1600 and 760 cm^{-1} which are indicative for a conjugated ketone and unsaturation, a hydroxyl band at 3600 cm^{-1} for a tertiary hydroxyl group. The UV spectrum showed absorptions at 234 and 290 nm indicating a dienone moiety. In the ^{1}H NMR spectrum, the observation of a singlet at δ 9.67, and an ion at m/z 438 $[M-30]^+$ in the EIMS supported the presence of the aldehyde group. Four methyl signals were observed for five methyl groups resonating at δ 0.82 (3H), 1.01 (3H), 1.13 (3H) and 1.19 (6H) as singlets. The presence of two olefinic protons were observed at δ 6.60 (s) and 5.40 (t, J = 2.5 Hz). The latter signal was characteristic for C-12 olefinic protons in olean and ursane triterpenes [9–10], indicating the location of a double bond between C-12 and C-13. Observation of an additional olefinic proton as fairly downfield at δ 6.60 indicated that it should be conjugated with both an oxo group and the double bond at C_{12}–C_{13}, therefore, the only place for the second double bond in the skeleton was between C-14 and C-15. The locations of the hydroxyl, carboxyl and aldehyde groups were assumed to be on the D or E ring which followed from the mass fragments at m/z 192 and m/z 277 originating from retro Diels-Alder cleavage of the C and D rings. The location of the carboxyl group should be at C-17, based on the mass fragment ion at m/z 423 $[M-COOH]^+$ as indicated in the literature [17]. The ion at m/z 215 was observed by the loss of hydroxy and carbon dioxide groups from the ion at m/z 277, and subsequently, an ion at m/z 186 indicated the presence of the aldehyde group to be in ring E. The ^{13}C NMR spectrum, using the APT technique, revealed 27 signals corresponding to 29 carbon atoms consisting of five methyls, two aliphatic methines, two olefinic methines, nine aliphatic methylenes, eight quaternary carbon signals, and three carbonyl carbons. The carbonyls at δ 191.1, 183.5 and at 204.2 were attributed to the aldehyde, the carboxyl and the keto group, respectively. A HETCOR experiment exhibited the direct correlations between protons and carbons, and a COLOC experiment allowed the assignment of the carbons (Table 1), particularly, for the determination of the localities of the tertiary hydroxyl, carboxyl, aldehyde and dienone moieties. The signal at δ 6.60 (H-15) showed a two-bond correlation with C-16 (204.2), a three-bond correlation with C-17 (55.2) and a four-bond correlation with C-28 (183.5), attributing the signal at δ 204.2 to the keto and the signal at δ 183.5 to the carboxyl moieties. The two-bond correlations between H_2-19 and qua-



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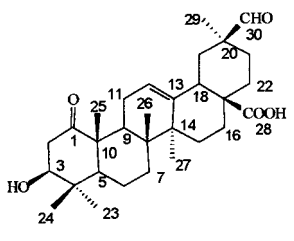

2

ternary carbon signals at δ 73.1 and δ 42.5 facilitated their assignment to C-18, and C-20, respectively. Therefore, the location of the tertiary hydroxy group was determined to be at C-18. Concerning the location of the aldehyde group, COLOC experiment was also very helpful giving two, three and four bond correlations between the aldehyde proton and C-20, with C-19 (C-21), and C-18, respectively (Table 1). Considering ^{13}C chemical shift of C-29 methyl group at δ 27.0, the streochemistry of aldehyde group was deduced to be β [18]. Since an interaction followed between C-18 OH and the aldehyde proton by a NOESY experiment C-18 OH would be also at β-position. Based on the above spectroscopic data, the structure of compound **1** was elucidated as 18-hydroxy-27-norolean-12,14-dien-30-al-28-oic acid.

The spectral data, particularly ^1H NMR spectrum of the second new compound **2** were similar to those of vergatic acid which was first obtained from *Salvia virgata* [19]. The HRMS gave a molecular ion peak at m/z 484.3211 corresponding to a molecular formula of $C_{30}H_{44}O_5$, having 9 degrees of unsaturation, which were accounted for by a pentacyclic ring system, one double bond, and three carbonyl groups. The IR spectrum supported the presence of three carbonyl bands observing at 1735, 1720 and 1690 cm^{-1} which indicated an isolated keto, aldehyde and acid carbonyls, respectively. In the ^1H NMR spectrum, six methyl singlets were observed at δ 0.77, 0.79, 0.93, 0.99, 1.08 and 1.25. The aldehyde proton appeared as a broadened singlet at δ 9.32. The presence of a Δ^{12} double bond at δ 5.31 (J = 2.5 Hz), and a C-3 proton signal bearing a hydroxyl group at δ 3.20 (J = 5 and 10 Hz), are characteristic for olean-type triterpenes. The presence of an isolated keto group in the structure was indicated by the IR spectrum with an absorption band at 1735 cm^{-1}, and this was supported by the MS. Considering the chemical shifts and multiplicities of H-3 and H-12, the plausible positions of the oxo group were C-1, C-6, C-7, C-14, C-15, C-19, C-21 or C-22. However, rings D and E, as well as ring C, were easily eliminated for the location of the oxo group following RDA fragment ions at m/z 222, and 204. Therefore, the remaining localities for the oxo group were only C-1, C-6 and C-7. The most useful information was obtained from the ^{13}C NMR spectrum, and COLOC experiment (Table 1), which exhibited three bond correla-

Table 1: NMR data of compounds 1 and 2 ($CDCl_3$)

	1 ^1H	**1** ^{13}C	COLOC	**2** ^1H	**2** ^{13}C	COLOC
1α	0.78 ddd	38.6	C-10, C-9, C-5	–	211.4	
1β	1.56 m		C-10, C-5	–		
2α	1.36 m	21.2	C-3, C-1, C-4	2.20 dd	44.1	C-3, C-1,C-10
2β	1.58 m		C-3, C-1, C-4	3.08 dd		C-3, C-1
3α	1.12 ddd	42.4	C-23, C-24, C-1	3.20 dd	78.6	C-2, C-1, C-4
3β	1.34 m		C-23, C-24, C-2	–		C-2, C-1, C-24
4	–	34.2		–	39.3	
5α	0.89 dd	53.1	C-6, C-7, C-23, C-24	1.68 m	54.0	C-4, C-6, C-7
6α	1.60 m	21.1	C-5, C-8	1.60 m	17.8	
6β	1.43 ddd		C-5, C-8	1.46 m		
7α	1.36 m	36.1	C-5, C-26	1.43 m	32.5	C-5, C-6, C-8, C-26
7β	2.12 m		C-26	2.15 m		
8		41.1		–	42.0	
9α	1.56 m	49.1	C-8, C-10, C-11	1.52 m	39.1	
10		41.0		–	52.3	
11α	1.92 m	25.6	C-10, C-9, C-12	1.95 m	25.3	C-9, C-12
11β	2.12 m		C-9, C-12	1.93 m		C-13
12	5.40 t	130.5	C-11, C-9, C-13, C-18, C-14	5.31 t	123.0	C-9, C-11, C-13, C-14
13	–	147.3		–	143.2	
14	–	163.6		–	39.4	
15α	6.60 s	146.5	C-13, C-14, C-16, C-17,C-28	1.02 m	25.5	C-14, C-16
15β	–			1.87 m		
16α	–	204.2		2.0 m	22.0	C-15, C-17, C-28
16β	–			0.88 m		
17	–	55.2		–	46.6	
18β	–	73.1		1.66 dd	42.5	
19α	2.40 d	50.1	C-18, C-20, C-30	1.47 m	40.1	
19β	2.52 d			1.35 m		
20	–	42.5		1.50 m	42.5	
21α	1.44 m	28.6	C-20, C-29, C-30	1.40 m	28.7	
21β	1.75 m			1.80 m		C-20, C-22,C-30
22α	2.48 m	35.4	C-28, C-29, C-30	2.60 m	35.7	C-17, C-21, C-28
22β	2.32 m		C-28, C-30, C-18	2.20 m		
23	1.01 s	27.3	C-24, C-5, C-3, C-4	0.93 s	28.5	C-24, C-4, C-5
24	1.19 s	16.1	C-23, C-4, C-5	0.79 s	16.0	C-23, C-4, C-5, C-3
25	1.13 s	16.5	C-9, C-5, C-1, C-4	0.99 s	15.0	C-9, C-10, C-1
26	0.82 s	27.3	C-9, C-14	0.77 s	17.5	C-9, C-8, C-14
27	–	–		1.08 s	25.7	C-13, C-14, C-15
28	–	183.5			183.1	
29	1.19 s	27.0	C-20, C-30, C-19, C-21	1.25 s	28.4	C-20, C-30
30	9.67 br s	191.1	C-20, C-29, C-19, C-21	9.32 br s	190.2	C-20, C-29

^1H NMR J (Hz) for compound **1:** 1α,1β = 13; 1α,2β = 13.5; 1α,2α = 2.5; 3α,2β = 12; 3α,2α = 3.5; 5α,6β = 12; 5α,6α = 2.5; 6α,6β = 14, 6β,7α = 13; 11α,12 = 11β,12 = 3; 19α,19β = 12, (18β-OH at 4.82 br s), for compound **2:** 2α,3α = 5; 2α,2β = 13.5; 2β, 3α = 10; 11α,12 = 11β,12 = 2.5; 18,19α = 11; 18,19β = 3

Table 2: Evaluation of cytotoxic potential[a]

Compd.	BC1	LU1	COL-2	KB	KB-V	P-388	LNCaP	ASK
1	>20	17.5	11.1	>20	>20	>5	15.7	–
2	>20	>20	>20	>20	>20	>5	>20	–
5 + 6	>20	17.7	>20	>20		>5	>20	–
9	10.4	7.5	9	>20	10.5	1.3	8.2	–
11 + 12	>20	>20	>20	>20	>20	>5	>20	–
13	>20	>20	>20	>20	>20	>5	>20	–
16 + 17	>20	>20	>20	>20	>20	>5	>20	–
Chloroform extract	>20	>20	>20	>20	>20	1.2	>20	–
Ellipticine (positive control)	0.2	0.02	0.3	0.04	0.3	0.1	0.8	–

[a] Compounds were initially tested at a concentration of 20 μg/ml, and this was followed by dose-response studies, as required, to yield ED_{50} values (μg/ml). With cultured ASK cells, tests were performed at a concentration of 20 μg/ml; colchicine was used as a positive control

tions between the keto carbonyl (211.4 ppm) and H-3 (δ 3.20), and two bond correlations between the keto carbonyl and CH_3-25, indicating the keto group should be at C-1. The location of the carboxyl group at C-17 followed from the MS which demonstrated a prominent fragment ion at m/z 440. Also, fragment ions at m/z 262, 218 and 189 arising from RDA cleavage were attributed to the location of acid and aldehyde groups to be in rings D or E. The correlation of the aldehyde carbon (190.2) with H-19, H-20 and H-21 in the COLOC experiment, was very indicative of the location of the aldehyde moiety to be at C-20. An interaction between the aldehyde proton and H-18β was clearly observed by a 2 D NOESY experiment [18, 20]. Thus, the structure of compound **2** was identified as 3β-hydroxy-1-oxo-olean-12-ene-30-al-28-oic acid. (vergatic acid-30-al = 1-oxo-dodecandral) [21].

Due to cytotoxic activity of betulinic acid and ursolic acid and their derivatives the compounds **1**, **2**, **5**, **6**, **9**, **11**, **12**, **13**, **16** and **17** isolated in sufficient amount and the chloroform extract were evaluated for cytotoxicity against a number of cell lines (see Table 2). While the chloroform extract was found to be highly active against P-388 (1.2 μg/ml), compound **1** showed a weak cytotoxic response against human colon cancer (11.1 μg/ml), hormone-dependent human prostate cancer (15.7 μg/ml), and human lung cancer (17.5 μg/ml). Among the tested compounds, 11-oxo-β-amyrin (**9**) showed a general cytotoxic response against all cell lines, except KB, and was particularly active against P-388 (1.3 μg/ml). None of the compounds gave a positive response with the ASK cell line, indicating a lack of antimitotic potential.

3. Experimental

3.1. General procedures

The spectra were recorded with the following instruments. IR: Perkin-Elmer 980 in $CHCl_3$; NMR: Bruker AC-200 L, 200 MHz and 50.32 MHz for ^1H and ^{13}C NMR respectively, and Bruker DPX 400, in $CDCl_3$; MS: ZabSpec high resolution mass spectrometer; CC: Silica gel 60 was used for column chromatography and Kieselgel $60F_{254}$ (E. Merck) for prep TLC with precoated plates.

3.2. Plant material

The plant material of *Lavandula stoechas* ssp. *stoechas* was collected from Gebze in Turkey, April 1996. The plant was identified and a voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Ankara University (AEF 19855).

3.3. Extraction and isolation

The powdered roots of the plant (800 g) were extracted with chloroform to yield 16 g of material. The extract was fractionated on a silica gel column (5 cm × 70 cm) eluting with hexane, followed by gradients with increasing amounts of chloroform, after 100% $CHCl_3$, elution was continued with increasing amounts of acetone, then methanol. Compound **1** was isolated during elution with CH_2Cl_2 : CH_3OH (75 : 25) and compound **2** during elution with CH_2Cl_2 : CH_3OH (98 : 2), and then purified by prep. TLC using solvent systems (CH_2Cl_2 : acetone) (50 : 50) for **1** and (CH_2Cl_2 : acetone) (95 : 5) for **2**, and obtained 23 mg and 18 mg, respectively. Among the known compounds, **16** and **17** were isolated from (hexane : CH_2Cl_2) (1 : 2) while **9** from 100% CH_2Cl_2. The other known compounds were isolated during the elution of CH_2Cl_2 : CH_3OH in increasing polarities: Compounds **7**, **8**, and **10** from CH_2Cl_2 : CH_3OH (99 : 1), **14** from CH_2Cl_2 : CH_3OH (98 : 2) **3** from CH_2Cl_2 : CH_3OH (97 : 3), **4** from (CH_2Cl_2 : CH_3OH) (96 : 4), **11, 12** and **15** in (25 : 75), **5**, **6** and **13** from (50 : 50). The all known compounds were identified based on spectral data (^1H NMR and EIMS) and melting points which were in agreement with literature values.

3.3.1. 18-Hydroxy-27-norolean-12,14-dien-30-al-28-oic acid (**1**)

$[\alpha]_D^{25} = +47.2°$ (c = 0.05); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3640 (OH), 1720 (CHO), 1685 and 2500 (sh) (COOH); 1660 (conjugated C=O), 1600 and 760 (unsaturation); ^1H NMR (200 MHz, $CDCl_3$) (see Table 1); EIMS (rel. int) at m/z: 468.3 $[M]^+$ (59), 450.3 $[M-H_2O]^+$ (33), 438.3 $[M-COH]^+$ (19), 435.3 $[450\text{-}CH_3]^+$ (24), 423.3 $[M-COOH]^+$ (30), 422 (15), 407.3 $[422\text{-}CH_3]^+$ (26), 277.2 $^+$(19), 248.1 (47), 215.1 (36), 203.1 (56), 186.1 $[215\text{-}CHO]^+$ (37), 146.1 (100), 133.1 (53), 119.1 (46), 107.1 (34), 95.1 (32), 81.1 (30), 69.1 (25). HRMS: 468.2890 for $C_{29}H_{40}O_5$ (calcd. 468.2875); ^{13}CNMR: see Table 1.

3.3.2. 3β-Hydroxy-1-oxo-olean-12-ene-30-al-28-oic acid (vergatic acid-30-al=1-oxo-dodecandral) (**2**)

$[\alpha]_D^{25} = +27.4°$ (c = 0.04); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (OH), 3000, 2980 (CH), 1735 and 1720, 1690 (C=O), 1685 (2500 sh); ^1H NMR (200 MHz, $CDCl_3$) (see Table 1); EIMS (rel. int) at m/z: 484.4 $[M]^+$ (14), 440 $[M-CO_2]^+$ (5), 422.3 $[M-CO_2]^+$ (15), 407.3 (19), 276.3 (2), 262.2 (8), 248.2. (30), 222.2 (21), 218.2 (42), 204.2 $[222\text{-}H_2O]^+$ (100), 189.1 $[218\text{-}CHO]^+$ (53), 175.1 (55), 133.1 (53), 119.1 (45), 107.1 (38), 95.1 (42), 81.1 (30), 69.1 (21). HRMS: 484.3211 $C_{30}H_{44}O_5$ (calcd. 484.3188); ^{13}C NMR: see Table 1.

3.4. Evaluation of cytotoxic activity

The $CHCl_3$ extract and compounds **1**, **2**, **5**, **6**, **9**, **11**, **12**, **13**, **16** and **17** were evaluated against cultured KB (human epidermoid carcinoma), BC1 (human breast cancer), LU1 (human lung cancer), COL-2 (human colon cancer), KB-V (+VLB) (drug-resistant KB), P-388 (mouse leukemia), LNCaP (hormone-dependent human prostate cancer), and ASK (rat glioma) cell lines [22].

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