Structure and Absolute Configuration of New Diterpenes from *Lavandula multifida*[⊥]

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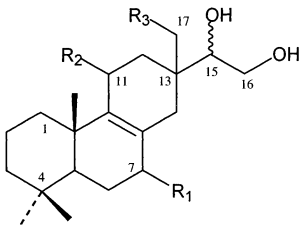
Four new diterpenes (**1**−**4**) have been isolated from the aerial parts of *Lavandula multifida*, together with the known compound glutinosin (**5**). The structures of these compounds were identified on the basis of extensive NMR studies as 15,16-dihydroxy-7,11-dioxopimar-8(9)-ene (**1**), 15S,16-dihyroxy-7-oxopimar-8(9)-ene (**2**), 15,16,17-trihydroxy-7-oxopimar-8(9)-ene (**3**), and 15,16,17-trihydroxypimar-8(9)-ene (**4**). The absolute configuration of the 15,16-diol moiety in **1**−**5** was determined observing the circular dichroism induced after addition of dimolybdenum tetracetate in DMSO solution.

We started a classical phytochemical study of *Lavandula multifida* L. (Labiatae), a Mediterranean species called Egyptian or fernleaf lavender,[1] whose aerial parts were collected in the south of Morocco by a Berber indigenous that used it for stomach pain. The genus *Lavandula* is commonly known for its essential oil. In fact the essence of some species has been traditionally employed in cosmetics and as sedative and antispasmodic remedies.[2,3] Several *Lavandula* species have already been chemically investigated, leading to the isolation of coumarins, triterpenes,[4] sesquiterpenes,[5] and several phenolic compounds as secondary metabolites.[6]

In this paper we report the isolation and structural characterization of four new diterpenes (compounds **1**−**4**), together with the known derivative glutinosin (**5**), belonging to the pimarane class, with the 1,2-dihydroxyethyl substitution at C-13. The absolute configuration of the 15,-16-diol moiety of **1**−**5** was determined observing the circular dichroism induced after in-situ complexation with dimolybdenum tetracetate in DMSO solution (Snatzke's method).

The EtOH extract of the aerial parts (20 g) of *L. multifida* was chromatographed over Sephadex LH-20 followed by HPLC to give compounds **1**−**5**. The molecular formula of compound **1** ($C_{20}H_{30}O_4$) was determined by ESIMS and elemental analysis. The UV spectrum (λ_{max} 275 nm) confirmed the presence of the α,β-unsaturated carbonyl group. The ^{1}H NMR spectrum (Table 1) showed signals for four tertiary methyl groups, a pair of doublets centered at δ 2.59 and 2.41 (J = 15.0 Hz) for the hydrogens of C-14, and signals for carbinolic protons at δ 3.40, 3.50, and 3.71. The ^{1}H NMR spectral data combined with 1D TOCSY and DQF-COSY experiments suggested the sequence C-1−C-3, C-5−C-6, C-15−C-16. The ^{13}C NMR spectrum of **1** (Table 2) showed the presence of two keto groups, a double bond, and a diol group. ^{13}C NMR signals were assigned on the basis of direct 2D ^{1}H−^{13}C experiments (HSQC). Locations of the carbonyl groups, the double bond, and the diol were confirmed by analysis of the HMBC experiment (Table SI, Supporting Information). In fact, the signal at δ 3.40 (H-15) correlated with carbon resonances at δ 51.0 (C-12), 31.0 (C-14), and 20.5 (C-17), permitting us to locate the diol group at C-15 and C-16; the signal at δ 2.50 (H-6) correlated with 34.0 (C-4), 141.5 (C-8), 200.4 (C-7), and 39.4 (C-10), and the signal at δ 1.36 (Me-20) correlated with 37.3 (C-1), 154.9 (C-9), and 201.6 (C-11), confirming the keto functions at C-7 and C-11. 1D ROESY and 2D NOESY measurements supported the proposed structure and proved the relative stereochemistry at C-5, C-10, and C-13. Thus, irradiation of the proton at δ 1.36 (Me-20) affected the Me-18 and H-15 signals, that of the proton signal at δ 1.72 (H-5) affected the Me-17 and Me-19 signals, while that of the proton at δ 0.90 (Me-17) weakly influenced Me-19 (see Figure 1). Consequently **1** was established as 15,16-dihydroxy-7,11-dioxopimar-8(9)-ene.



	R_1	R_2	R_3	C-17
1	=O	=O	–H	β
2	=O	–H	–H	β
3	=O	–H	–OH	β
4	–H	–H	–OH	β
5	–H	–H	–H	α

The ESIMS of compound **2** ($C_{20}H_{32}O_3$) showed a molecular ion peak at *m*/*z* 320 that differed from that of compound **1** by 14 mu. The UV spectrum confirmed the presence of an α,β-unsaturated carbonyl group. Comparison of NMR spectra of **1** and **2** indicated structural similarities; the main differences were the absence of one signal for a carbonyl group and the presence of an additional methylene signal in the ^{13}C and ^{13}C DEPT NMR spectra (Table 1). The upfield shift of C-8, C-13, and C-12

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⊥ Dedicated to the memory of Prof. Serena Catalano.

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**4** (600 MHz, CD_3OD)[a]

proton	1	2	3	4
1a	1.20	1.30	1.36	1.20
1b	1.80	1.85	1.93	1.82
2a	1.75 dd (12.0, 5.0)	1.65	1.75	1.75
2b	1.56	1.57	1.61	1.56
3a	1.48	1.50	1.52	1.46
3b	1.28	1.23	1.28	1.19
5	1.72	1.74 dd (12.0, 5.0)	1.80	1.19
6a	2.55 d (1.1)	2.54 dd (12.0, 5.0)	2.50 br s	1.46
6b	2.50 d (8.2)	2.40 (12.0, 3.0)	2.47 d (5.3)	1.50
7a				1.97
7b				2.01
11a		2.22	2.33 d (17.0)	1.98
11b		2.38	2.37 m	2.06
12a	2.11 dd (14.0, 1.7)	1.60	1.65	1.61
12b	2.83 d (14.0)	1.41	1.59	1.46
14a	2.59 d (18.0)	2.15	2.07 d (17.0)	1.63
14b	2.41 dd (18.0, 1.2)	2.23	2.30 br d (13.0)	2.08 d (17.0)
15	3.40 dd (8.0, 3.4)	3.50 dd (10.0, 2.5)	3.55 dd (7.0, 2.6)	3.63
16a	3.50 dd (11.4, 8.0)	3.61 dd (11.0, 2.5)	3.66 dd (11.8, 7.0)	3.69 dd (10.0, 5.5)
16b	3.71 dd (11.4, 3.2)	3.75 dd (11.0, 10.0)	3.72 dd (11.8, 3.1)	3.71 dd (10.0, 3.0)
17a	0.90 s	0.83 s	3.50 d (11.4)	3.50 d (11.0)
17b			3.36 d (11.4)	3.47 d (11.0)
18	0.93 s	0.91 s	0.94 s	0.92 s
19	0.96 s	0.94 s	0.99 s	0.98 s
20	1.36 s	1.12 s	1.16 s	1.05 s

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in δ units.

Table 2. ^{13}C NMR Spectral Data (δ) for Compounds **1**−**4** (600 MHz, CD_3OD)

carbon	1	2	3	4
1	37.3	37.0	36.8	38.3
2	19.5	19.8	19.4	19.6
3	41.8	42.5	42.0	42.8
4	34.0	34.4	33.8	33.8
5	50.8	52.2	51.3	53.0
6	36.0	36.8	36.2	20.6
7	200.4	200.4	200.0	33.0
8	141.5	128.5	129.0	124.0
9	154.9	167.0	168.1	138.4
10	39.4	37.2	40.5	38.2
11	201.6	23.0	22.5	20.6
12	51.0	31.6	26.4	27.9
13	37.8	35.5	40.5	41.0
14	31.0	32.2	28.4	36.8
15	78.3	78.3	76.7	77.0
16	63.9	63.9	63.6	64.0
17	20.5	21.0	65.7	67.3
18	32.8	33.9	32.6	33.0
19	21.0	22.6	21.5	20.5
20	17.0	20.0	18.4	20.3

and the downfield shift of C-9 in the ^{13}C NMR spectrum indicated the absence of a carbonyl group at C-11. Direct evidence for the proton sequence and substituent sites was derived from HSQC and HMBC experiments (Table SI, Supporting Information). The relative stereochemistry of **2** was determined on the basis of the results of 1D ROESY and NOESY experiments that were in good agreement with those of **1**. Thus, compound **2** was determined to be 15,-16-dihydroxy-7-oxopimar-8(9)-ene.

Compound **3** had the molecular formula $C_{20}H_{32}O_4$ as determined by ^{13}C, ^{13}C-DEPT NMR, and ESIMS experiments. The ^{13}C and ^{13}C-DEPT NMR indicated the same diterpene skeleton as compounds **1** and **2**. In the ^{1}H NMR spectrum, the presence of an α,β-unsaturated ketone was inferred from the double doublet at δ 2.47 and 2.50 for H_2-6 and was confirmed by the chemical shifts of H_2-14 and H_2-11. Results of 1D TOCSY, DQF-COSY, HSQC, and HMBC experiments allowed us to assign the entire ^{1}H and ^{13}C spectra. 1D ROESY measurements supported the proposed structure and revealed the relative stereochemistry at C-5,

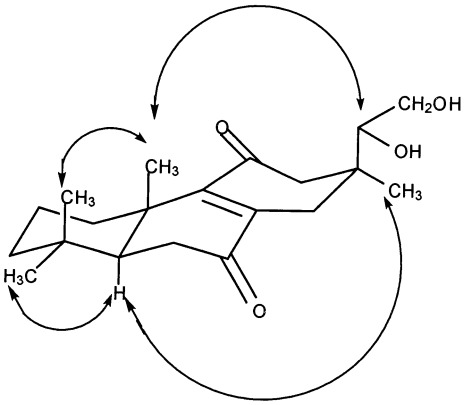

Figure 1. NOESY correlations of compound **1**.

C-10, and C-13. Thus irradiation of the proton at δ 1.16 (Me-20) affected Me-18 and H-15, while irradiation at δ 3.55 (H-15) affected the signal of Me-18. Consequently compound **3** was established as 15,16,17-trihydroxy-7-oxopimar-8(9)-ene.

The ESIMS of compound **4** ($C_{20}H_{34}O_3$) showed the quasi-molecular peak at *m*/*z* 345 $[M + Na]^+$. Analysis of NMR and UV spectral data of compound **4** and comparison with those of **3** showed that **4** differed from **3** only by absence of the carbonyl group at C-7 (Tables 1 and 2). Therefore **4** was identified as 15,16,17-trihydroxypimar-8(9)-ene.

Compound **5** was assigned the molecular formula $C_{20}H_{34}O_2$. The mass spectral data, optical rotation, and ^{1}H and ^{13}C NMR data of **5** were in good agreement with those of 15,16-dihydroxyisopimar-8(9)-ene, known as glutinosin.[7]

The relative stereochemistry of the several centers on the rings can be determined through NMR, owing to the structural rigidity. On the contrary, the sidearm is flexible and attached to the rest of the molecule through a quaternary carbon, which prevents the use of ^{1}H−^{1}H coupling constants. An empirical correlation exists between the ^{13}C chemical shift of pimarane C-15 in pimarenes and the configuration of this center: it has been reported that (15*R*)-15,16-dihydroxy pimaranes have $\delta(^{13}\text{C-15}) \approx 76$ ppm, while 15*S* epimers have $\delta(^{13}\text{C-15}) \approx 78$ ppm.[8] Such a

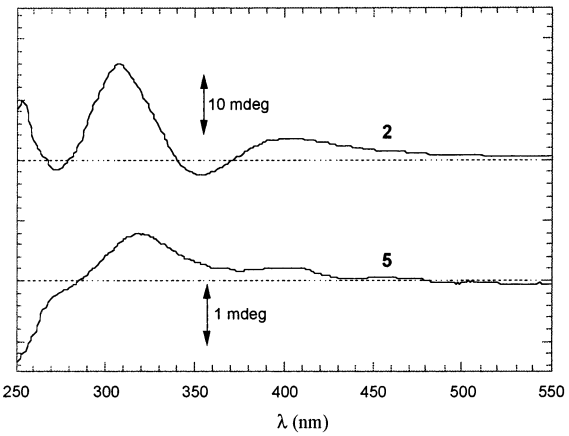

Figure 2. Circular dichroism spectra of **2** (top) and **5** (bottom) in a DMSO solution of dimolybdenum tetracetate. For **2** the inherent CD of the diol was subtracted.

relation is seriously limited by the following observations: the difference in shift between the two epimers is small; it has been tested on a restricted number of compounds; it has been used only for pimarane and *ent*-pimarane skeletons, while little is known for *iso*-pimaranes.[9] Indeed, from the analysis of the data reported in Table 1, it is clear that the transition between the two δ values may not be sharp, and moreover the role of the chirality of the nearby C-13 is not clear. The problem of assigning the absolute configuration of the 1,2-dihydroxyethyl group can be solved by means of CD methods, provided that the following points are taken into account: the 1,2-diol moiety is CD-inactive above 190 and needs derivatization with suitable chromophoric groups; its conformational flexibility must be reduced to make the CD information profitable for a configurational assignment; interference from the unsaturated chromophores (especially when conjugation is extended as in **1**−**3**) must be either taken into account or avoided; the small quantity of products available seriously limits the chemical handling. All these requisites are fulfilled employing in-situ complexation with dimolybdenum tetracetate, one of the most reliable methods for assigning the absolute configuration of acyclic 1,2-diols.[10,11]

Two samples (**2** and **5**) were amenable to CD analysis with dimolybdenum tetracetate. According to the rule proposed by Snatzke, which found no real exceptions in the literature,[10] the sign of the diagnostic band at about 305 nm is correlated to the absolute configuration of the chiral centers in the 1,2-diol moiety. In particular an *S*-monosubstituted glycol gives rise to a positive Cotton effect at 305 nm. Thus, the positive sign observed in the spectra shown in Figure 2 allowed us to assign the *S*-configuration to C-15 in both products **2** and **5**. Unfortunately, the other compounds were not available in sufficient quantity and purity for this analysis. Compound **2** has an inherent CD, which overlaps the one resulting after addition of dimolybdenum tetracetate. Thus, the inherent contribution must be subtracted to obtain the induced CD of the inorganic complex, which is correlated to the absolute configuration of the diol. This procedure is justified by the negligible interaction between backbone and dimolybdenum transitions.

Although the power of NMR methods to elucidate structures of natural products not amenable to diffractometric analyses is unquestionable, there may be cases when they are not sufficient. In the present report, we demonstrated that without resorting to chemical correlation or dealing with crystalline material, it was possible to recognize the pimarane and *iso*-pimarane skeletons of the diterpenes isolated from *Lavandula multifida*. Nonetheless, owing to the flexibility of the dihydroxyethyl arm and the connection to the backbone through a quaternary center, the determination of chirality at C-15 could lead to a serious problem. Indeed, in recent work on similar structures, this point has been left open.[12] We demonstrated that with the application of the simple and straightforward Snatzke's chiroptical method it could be possible to determine the stereochemistry of the diol arm independently of the rest of the molecule. It is noteworthy that no interference from the unsaturated ketone in **2** is observed, in agreement with what was previously reported on this method.[10,11]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Perkin-Elmer-Lambda 11 spectrophotometer. A Bruker DRX-600 NMR spectrometer was employed, operating at 599.19 MHz for ^{1}H and 150.86 MHz for ^{13}C, using the UXNMR software package; chemical shifts are expressed in δ (ppm) referring to the residual solvent peaks δ_H 3.34 and δ_C 49.0 for deuterated MeOH. ^{13}C-DEPT, 1D TOCSY, ^{1}H−^{1}H DQF-COSY, 1D ROESY, 1D NOESY, ^{1}H−^{13}C HSQC, and HMBC experiments were carried out using the conventional pulse sequences as described in the literature.[13] ESIMS (positive mode) were obtained from a Finningan LC-Q Deca Termoquest spectrometer, equipped with Excalibur software. Column chromatography was performed over Sephadex LH-20 (Pharmacia); HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and with LiChrospher 100 DIOL and LiChrospher 100 RP-18 columns and a Shimadzu injector. CD spectra were measured on a JASCO J-715 spectropolarimeter with a 0.1 cm cell in DMSO at room temperature with the following conditions: speed 50 nm/min, time constant 1 s, bandwidth 2.0 nm; noise reduction was carried out with a low-pass filter.

Plant Material. The aerial parts of *L. multifida* were collected in the South of Morocco, in December 1999. The plant material was identified by Prof. Paolo Emilio Tomei, of Dipartimento di Agronomia e Gestione dell'Agroecosistema (DAGA), Università di Pisa, where a voucher specimen was deposited.

Extraction and Isolation. The air-dried powdered aerial parts of *L. multifida* (20 g), collected in the South of Morocco, in December 1999, were macerated with EtOH at 90 °C. The extract was evaporated under reduced pressure, yielding 3.5 g of residue, which was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain 42 fractions of 8 mL, which were pooled into eight major fractions. Fraction 3 was purified by HPLC on a LiChrospher 100 DIOL column (25 cm × 1.5 cm, flow rate 2 mL min^{-1}) using $CHCl_3$ as eluent to obtain compound **2** (t_R = 17 min, 5.1 mg) together with another fraction that was further fractionated over RP-HPLC on a LiChrospher 100 RP-18 column (25 cm × 4.6 mm, flow rate 0.8 mL min^{-1}) with MeOH−H_2O (60:40) to yield compound **3** (t_R = 25 min, 1.2 mg). Fraction 4, from the initial purification over Sephadex LH-20, was chromatographed over HPLC on a LiChrospher 100 DIOL column (25 cm × 1.5 cm, flow rate 2 mL min^{-1}), with $CHCl_3$ as eluent to afford compound **5** (t_R = 10 min, 4.0 mg) together with two other major fractions, A and B, that were further fractionated over RP-HPLC on a LiChrospher 100 RP-18 column (25 cm × 4.6 mm, flow rate 0.8 mL min^{-1}) with MeOH−H_2O (75:25) (fraction A) and with MeOH−H_2O (70:30) (fraction B), to give, respectively, compound **1** (t_R = 16 min, 1.0 mg) from fraction A and compound **4** (t_R = 35 min, 2.0 mg) from fraction B.

15,16-Dihydroxy-7,11-dioxopimar-8(9)-ene (1): colorless oil; $[\alpha]_D^{25}$ −14.4° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 275

(4.06) nm; ^{1}H NMR (600 MHz, CD_3OD), see Table 2; ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m/z* 691 $[M + Na + M]^+$, 357 $[M + Na]^+$, 339 $[M + Na - 18]^+$, 297 $[M + Na - 60]^+$; *anal.* C 71.70%, H 9.08%, O 19.22%, calcd for $C_{20}H_{30}O_4$, C 71.82%, H 9.05%, O 19.13%.

15*S*,16-Dihydroxy-7-oxopimar-8(9)-ene (2): colorless oil; $[\alpha]_D^{25}$ +11.8° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (2.16) nm; ^{1}H NMR (600 MHz, CD_3OD), see Table 2; ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m/z* 343 $[M + Na]^+$, 325 $[M + Na - 18]^+$; *anal.* C 74.90%, H 10.08%, O 15.02%, calcd for $C_{20}H_{32}O_3$, C 74.96%, H 10.06%, O 14.98%.

15,16,17-Trihydroxy-7-oxopimar-8(9)-ene (3): amorphous powder; $[\alpha]_D^{25}$ +12.9° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 250 (3.25) nm; ^{1}H NMR (600 MHz, CD_3OD), see Table 2; ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m/z* 359 $[M + Na]^+$, 341 $[M + Na - 18]^+$; *anal.* C 71.30%, H 9.62%, O 19.08%, calcd for $C_{20}H_{32}O_4$, C 71.39%, H 9.59%, O 19.02%.

15,16,17-Trihydroxypimar-8(9)-ene (4): amorphous powder; $[\alpha]_D^{25}$ +36.0° (*c* 0,1, MeOH); ^{1}H NMR (600 MHz, CD_3OD), see Table 2; ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m/z* 667 $[M + Na + M]^+$, 345 $[M + Na]^+$, 327 $[M + Na - 18]^+$; *anal.* C 74.45%, H 10.66%, O 14.89%, calcd for $C_{20}H_{34}O_3$, C 74.49%, H 10.63%, O 14.88%.

15S,16-Dihydroxyisopimar-8(9)-ene (5): colorless oil; $[\alpha]_D^{25}$ +32° (*c* 0.65, $CHCl_3$); ^{1}H NMR and ^{13}C NMR in agreement with glutinosin;[7] ESIMS *m/z* 329 $[M + Na]^+$, 311 $[M + Na - 18]^+$; *anal.* C 78.35%, H 11.17%, O 10.48%, calcd for $C_{20}H_{34}O_2$, C 78.38%, H 11.18%, O 10.44%.

Determination of Absolute Configuration of the 15,-16-Diol Moiety Using Snatzke's Method. Dimolybdenum tetracetate was purchased from Fluka. DMSO, spectroscopy grade, was obtained from Fluka and dried according to the common procedure. According to the published procedure,[11] about 1:1 diol-to-molybdenum mixtures were prepared using 0.7 mg/mL of compounds **1**−**5**. Soon after mixing, the first CD spectrum was recorded and its evolution monitored until stationary (30−60 min). The sign of the diagnostic band at 305 nm (band IV, according to Snatzke's nomenclature) is correlated to the absolute configuration of the 15,16-diol moiety. For **2**, the inherent CD of the diol was subtracted.

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Supporting Information Available: HMBC correlations observed for compounds **1**−**4**, Table SI. This material is available free of charge via the Internet at http://pubs.acs.org.

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