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Necrodane monoterpenoids from *Lavandula luisieri*

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Abstract

Four new irregular monoterpenoids were identified in the essential oil of *Lavandula luisieri* (Rozeira) Riv. Mart., a Labiatae endemic to the Iberian Peninsula. By means of spectroscopic and chemical methods, they were characterised as 3,5-dimethylene-1,4,4-trimethylcyclopentene, 5-methylene-2,3,4,4-tetramethylcyclopent-2-enone, 3,4,5,5-tetramethylcyclopenta-1,3-dienecarboxaldehyde and 3,4,5,5-tetramethylcyclopenta-1,3-dienecarboxylic acid. *L. luisieri* essential oil and extract showed a good activity against *Candida albicans* and gram positive bacteria: *Staphylococcus aureus*, *S. epidermidis* and *S. pyogenes*.

Keywords: *Lavandula luisieri*; Labiatae; Necrodane monoterpenoids; Antibacterial and antifungal activities

1. Introduction

Lavandula luisieri (Rozeira) Riv. Mart. is an aromatic Labiatae widespread in the southwest of the Iberian Peninsula. Some authors (Upson and Andrews, 2004) classified recently this plant as a subspecies of *L. stoechas* L. as it is morphologically nearly identical to *L. stoechas* L. subsp. *stoechas*. However, the nature of the volatile components of the two plants is significantly different. Obviously, these species are genetically well differentiated and their chemical composition can be considered as a reliable marker for their distinction. Although many studies were published on the chemical composition of the essential oil of *L. stoechas* L., which is constituted mainly by camphor and fenchone (Lawrence, 2004), few publications reported on the volatile compounds of *L. luisieri*. Garcia Vallejo et al. (1994) found that *L. luisieri* oil had an atypical composition, characterised by the presence of irregular monoterpenoids: *trans*-α-necrodol (**1**) and *trans*-α-necrodyl acetate (**2**) were the main constituents. Recently, Sanz et al. (2004) applied direct thermal desorption–gas chromatography–mass spectrometry for the analysis of volatile components of *L. luisieri* and identified *cis*-α-necrodyl acetate (**3**) in addition to **1** and **2** (Fig. 1).

In a recent article (Lavoine-Hanneguelle and Casabianca, 2004), we described the analysis of the essential oil and extract of *L. luisieri*. We wish to report here the structure elucidation of four new necrodane derivatives and thus to revise the stereochemistry of two compounds proposed in the previous communication. In addition, the antibacterial activity of the essential oil and extract was evaluated for the first time (see Fig. 2).

2. Results and discussion

The essential oil of the aerial parts of *L. luisieri* L. was distilled under vacuum and the fractions thus

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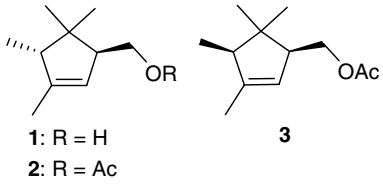

Fig. 1. Selected necrodane compounds.

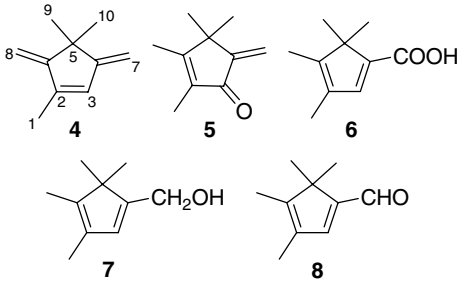

Fig. 2. Structure of some necrodane terpenoids.

obtained were analysed by GC–MS. Mass spectra and Kovat's retention indexes on an apolar column (HP1) were compared with a commercial library of mass spectra and with a laboratory-made library of authentic samples. Together with *trans*-α-necrodol (**1**), *trans*-α-necrodyl acetate (**2**) and 1,8-cineole, representing more than 50% of the total amount, the following compounds were identified in the essential oil: α-pinene, (*Z*)-β-ocimene, fenchone, linalool, camphor, myrtenyl acetate, γ-terpinene, trans linalool oxide, α-copaene, lavandulyl acetate, *allo*-aromadendrene, α-terpineol, *δ*-cadinene, selina-4,7(11)-diene, selina-3,7(11)-diene, epi-cubenol, *cis*-α-copaene-8-ol, viridiflorol, and *τ*-muurolol.

As several compounds could not be identified by this method, we performed the isolation and the structural analysis of the main ones from their MS, 1D and 2D NMR data. By repeated column chromatography on silica gel and silver nitrate impregnated silica gel, a monoterpene (**4**) and a monoterpenic ketone (**5**) were isolated. A necrodanic acid (**6**) was also obtained from the acidic part of the essential oil. In order to identify other necrodane compounds, some chemical conversions were applied to a pure sample of **6**. The $LiAlH_4$ reduction furnished the alcohol **7** which proved to be extremely unstable. However, it could be oxidized to aldehyde **8** by pyridinium chlorochromate. From the mass spectra and retention times of the synthetic samples of **7** and **8**, their presence in the essential oil was investigated. As expected, alcohol **7** was not detectable, but **8** was identified in the essential oil samples at an amount of ~1%.

2.1. 3,5-Dimethylene-1,4,4-trimethylcyclopentene (*4*)

The most volatile fraction of distillation was a mixture of α-pinene, 1,8-cineole and a third component presenting a mass spectrum identical to those of paracymene, but with a lower Kovat's retention index. After several successive chromatographic separations, this compound was isolated as a colourless oil with a pleasant lemon-like odour. In addition to the molecular ion at *m*/*z* 134 in the mass spectrum, the NMR data suggested a monoterpene with four degrees of insaturation, possessing a cyclopentenic structure bearing a gem-dimethyl moiety as well as two exocyclic methylenes and a methyl. Careful examination of the NOESY correlations (Fig. 3) enabled us to determine unambiguously the spatial disposition of these substituents on the ring and **4** was identified as the first necrodane monoterpene: 3,5-dimethylene-1,4,4-trimethylcyclopentene. This compound is relatively abundant in the essential oil (2–4%) and is not an artifact of the hydrodistillation as its presence was detected also in the extract (obtained by extraction at room temperature).

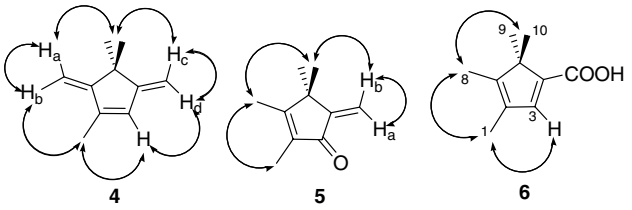

Fig. 3. NOESY correlations observed for compounds **4**–**6**.

2.2. 5-Methylene-2,3,4,4-tetramethylcyclopent-2-enone (*5*)

Compound **5** was isolated from the intermediate fraction by repeated column chromatography on silica gel. In our previous communication (Lavoine-Hanneguelle and Casabianca, 2004) the structure of this compound was proposed as 2,3,5,5-tetramethyl-4-methylen-2-cyclopenten-1-one on the basis of its monodimensional ^1H and ^{13}C NMR spectral data. However, while the cyclopenten-2-one moiety was easily deduced from this set of data, the relative position of the methylene and methyl substituents had to be confirmed by further investigations. The COSY, HSQC, HMBC and NOESY experiments helped us to establish the structure of **5** as 5-methylene-2,3,4,4-tetramethylcyclopent-2-enone (Fig. 3).

2.3. 3,4,5,5-Tetramethyl-1,3-cyclopentadienecarboxylic acid (*6*)

From the acidic part of the essential oil, a yellowish solid compound was isolated and purified by column chromatography. In our previous work, this compound was also mistakenly identified, as 2,4,5,5-tetramethyl-1,3-cyclopentadienecarboxylic acid, but the use of 2D NMR experiments led us to revise its stereochemistry

and **6** was finally characterised as 3,4,5,5-tetramethyl-1,3-cyclopentadienecarboxylic acid.

2.4. Synthesis of 3,4,5,5-tetramethyl-1,3-cyclopentadienecarboxaldehyde (*8*)

The presence of **6** in the essential oil led us to suspect that the corresponding aldehyde **8** could be its precursor. Indeed, **6** was invariably identified in samples of freshly distilled essential oil, but the acid number of the oldest samples was always higher than the fresher ones. To the best of our knowledge, compound **8** was never described in the literature. Then, we prepared it by $LiAlH_4$ reduction of **6** followed by PCC oxidation. After work-up and purification on flash chromatography, aldehyde **8** was isolated. From the MS spectral data and Kovat's retention index of this hemisynthetic sample, the presence of **8** in the essential oil was unambiguously confirmed. Surprisingly, the necrodane skeleton is extremely rare among the monoterpenoids: *trans*-α-necrodol was first discovered as a constituent of the defensive spray of a South American carrion beetle: *Necrodes surinamensis* (Eisner and Meinwald, 1982; Eisner et al., 1986; Roach et al., 1990), and up to now, *L. luisieri* is the only other natural source of necrodane derivatives. The presence of necrodane compounds in defensive secretions led us to suppose potential biological properties. Before a study on pure compounds, we decided to evaluate the global activity of the natural mixture itself: the antibacterial and antifungal activities of *L. luisieri* essential oil and extract were evaluated against several species of bacteria and fungi using the method described by Lorian (1996). The minimum inhibitory concentrations (MICs) are detailed in Table 3. The essential oil and extract show similar activities, which are significantly good against *Candida albicans* and gram positive bacteria: *S. aureus*, *S. epidermidis* and *S. pyogenes.*

3. Experimental

3.1. General

The NMR spectra were recorded on a Bruker WM 200 or 500 MHz spectrometer in $CDCl_3$. The chemical shift values are reported with reference to TMS and the coupling constants are given in Hz. GC–MS analyses were performed with a Hewlett–Packard 5890 chromatograph coupled with a 5970A mass spectrometer of the same company, using the following conditions: fused-silica capillary column HP-1 (dimethylsiloxane) 50 m × 0.2 mm i.d., film thickness 0.5 μm. Injection mode: split 1:30. The oven temperature was programmed from 60 to 250 °C at 2 °C/min and then held isothermal for 30 min. The carrier gas was He (0.8 ml/min). Injector and detector temperatures were 250 and 230 °C, respectively. The mass spectra were performed at 70 eV, mass range 35–400.

3.2. Plant material

The aerial parts of *L. luisieri* were collected in the region of Almaden de la Plata, near Seville, Spain, 2000–2003 and was identified under the supervision of MI Garcia Vallejo. A voucher specimen (voucher number B-5154) was deposited in the herbarium of the Muséum d'Histoire Naturelle de la ville de Nice.

3.3. Extraction and isolation

The extract was obtained by ethanolic extraction of the hexane extract produced from the aerial parts of the fresh flowering plant. The ethanolic solution thus obtained was cooled and filtered to remove waxes, and then concentrated at room temperature under vacuum. The extract yield was about 0.5% (w/w).

The essential oil of *L. luisieri* was obtained by hydrodistillation of the aerial parts of the plant on an industrial scale for 4 h. The oil yield was 0.2% (w/w), on fresh weight basis. The oil was then distilled under vacuum using a spinning band fractional distillation, leading to several fractions which were subjected to chromatographic separation in order to isolate and identify the unknown compounds.

3.4. 3,5-Dimethylene-1,4,4-trimethylcyclopentene (*4*)

Compound **4**: colourless oil, R_I = 924, EI-MS 70 eV *m/z* (rel. int.): 134 [M^+] (39), 119 (100), 115 (7), 91 (28), 77 (8), 65 (4), 51 (3), 41 (4), 39 (4). ^1H and ^{13}C NMR (see Tables 1 and 2). HREIMS 134.1106 (M^+, $C_{10}H_{14}$ requires 134.1096).

3.5. 5-Methylene-2,3,4,4-tetramethylcyclopent-2-enone (*5*)

Compound **5**: colourless oil, R_I = 1160, EI-MS 70 eV *m/z* (rel. int.): 150 [M^+] (54), 135 (66), 107 (100), 91 (39), 79 (21), 53 (16), 41 (16), 39 (12). ^1H and ^{13}C NMR (see Tables 1 and 2). HREIMS 150.1045 (M^+, $C_{10}H_{14}O$ requires 150.1045).

3.6. 3,4,5,5-Tetramethylcyclopenta-1,3-dienecarboxylic acid (*6*)

A sample of the whole essential oil (105 g) was added dropwise in a cold diluted solution of sodium carbonate (10 g in 400 ml) and stirred during 2 h. After decantation, the aqueous phase was extracted with 4 × 100 ml Et_2O, and neutralised slowly with diluted HCl until pH 3. The acidic aqueous phase was then extracted with 3 × 100 ml Et_2O, washed with brine, dried on $MgSO_4$

Table 1
^{1}H NMR spectral data of compounds **4**–**6** and **8**

Atom no.		**4**			**5**		**6**		**8**	
		δ ^{1}H	*m* (*J*)		δ ^{1}H	*m* (*J*)	δ ^{1}H	*m* (*J*)	δ ^{1}H	*m* (*J*)
1		1.91	*d* (1.55)		1.77	*d* (0.80)	1.86	*m*	1.92	*m*
2		–						–		–
3		6.20	*s*				7.24	*s*	7.08	*s*
4		–						–		–
5		–						–		–
6		–						–		–
7	a	4.74	*d* (1.51)	a	5.99	*s*	–	–	9.60	*s*
	b	4.86	*s*	b	5.27	*s*				
8	c	4.64	*s*		1.97	*d* (0.80)	1.79	*m*	1.83	*m*
	d	4.82	*s*							
9		1.11	*s*		1.22	*s*	1.16	*s*	1.18	*s*
10		1.11	*s*		1.22	*s*	1.16	*s*	1.18	*s*

Assignment of the protons and the corresponding carbons (Table 2) of compounds **4**–**6** was deduced from DEPT spectra and COSY, NOESY and HSQC NMR experiments.

Table 2
^{13}C NMR spectral data of compounds **4**–**6** and **8**

Atom no.	**4**	**5**	**6**	**8**
1	13.0	8.2	12.2	12.6
2	143.6	135.8	130.6	132.1
3	133.9	195.5	147.6	153.4
4	161.3	152.4	142.3	154.2
5	43.9	43.9	53.4	53.0
6	163.0	171.9	158.4	161.3
7	100.0	113.1	168.7	185.5
8	100.1	11.5	10.0	10.1
9	28.4	25.6	21.3	21.8
10	28.4	25.6	21.3	21.8

and evaporated to give 320 mg of a yellow solid. This product proved to be an almost pure sample of a compound detected in the chromatogram of the essential oil. It was then purified by column chromatography on silica gel to afford **6** (200 mg) as a white solid, R_I = 1269, EI-MS 70 eV *m*/*z* (rel. int.): 166 [M^+] (85), 151 (16), 133 (20), 121 (59), 107 (100), 105 (63), 91 (66), 79 (37), 77 (31), 51 (15), 39 (16). ^{1}H and ^{13}C NMR (see Tables 1 and 2). HREIMS 166.0987 (M^+, $C_{10}H_{14}O_2$ requires 166.0994).

3.7. Synthesis of 3,4,5,5-tetramethylcyclopenta-1,3-dienecarboxaldehyde (*8*)

A solution of **6** (100 mg) in 4 ml dry THF was added dropwise in a suspension of 151 mg $LiAlH_4$ in 8 ml dry THF cooled in an ice bath. At the end of the addition, the ice bath was removed and the stirring was maintained during 2 h. The reaction mixture was then poured in a solution of $NaHCO_3$ containing ice and CH_2Cl_2 (30 ml) under vigorous stirring. The mixture was quickly decanted, and the aqueous phase was extracted another time with 30 ml CH_2Cl_2. The combined organic phases were then washed with brine, dried with a minimal amount of $MgSO_4$ and evaporated under vacuum at room temperature to a volume of 3 ml. This solution of **7** was directly used in the next step without purification: it was added dropwise to a suspension of 161 mg of pyridinium

Table 3
Antibacterial and antifungal activities of *L. luisieri* essential oil and extract

Strains	Essential oil[a]	Extract[a]
Staphylococcus aureus ATCC 6538	1/160	1/160
Staphylococcus aureus ref Nr 1[b] (wild strain)	1/160	1/160
Staphylococcus aureus ref Nr 4[b] (wild strain)	1/160	1/160
Staphylococcus epidermis ATCC 12228	1/160	1/160
Staphylococcus epidermis ref Nr 131[b] (wild strain)	1/160	1/160
Enteroccocus hirae ATCC 10541	1/80	1/80
Enteroccocus faecalis ref Nr 5[b] (wild strain)	1/40	1/40
Streptococcus pyogenes CIP 5641	1/160	1/160
Bacillus subtilis ATCC 6633	1/80	1/160
Bacillus cereus ATCC 14893	1/20	1/20
Escherichia coli ATCC 8739	1/20	1/20
Salmonella typhimurium CIP60.62T	1/20	1/20
Klebsiella pneumoniae ATCC 10031	1/40	1/40
Enterobacter cloacae ATCC 23335	1/20	1/20
Serriata marcescens ATCC 13880	1/20	1/20
Pseudomonas aeruginosa ATCC 9027	1/20	1/20
Candida albicans ATCC 10231	1/160	1/160
Candida albicans ATCC 2091	1/160	1/160
Candida albicans ref Nr 1[b] (wild strain)	1/160	1/160
Candida albicans ref Nr 2[b] (wild strain)	1/160	1/160
Candida albicans ref Nr 3[b] (wild strain)	1/160	1/160
Candida albicans ref Nr 4[b] (wild strain)	1/160	1/160

[a] Results are expressed as the dilution ratio of the mixture.
[b] Strain developed in our laboratory.

chlorochromate in 50 ml of CH_2Cl_2 at room temperature. After 2 h of stirring, the mixture was poured in 150 ml of Et_2O and the resulting suspension was filtrated on glass wool. The limpid yellow solution was washed successively with 2×100 ml water and 2×100 ml brine, and evaporated to yield 21 mg of **8** as a colourless oil, $R_I = 1130$, EI-MS 70 eV *m/z* (rel. int.): 150 [M^+] (47), 135 (37), 122 (22), 107 (100), 105 (29), 91 (65), 79 (31), 77 (21), 65 (11), 51 (10), 41 (10), 39 (13). ^1H and ^{13}C NMR (see Tables 1 and 2).

3.8. Bioactivity evaluation

The oil and extract were first diluted at 50% by weight in a mixture 50:50 of methanol (RP Normapur® Prolabo) and Cremophor® El (BASF). Then the serial twofold dilutions were prepared in sterile water. MICs were determined by the twofold agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) for bacteria and with Sabouraud dextrose agar for fungi. The overnight cultures for bacteria and cultures of 48 h for fungi were diluted to approximately 10^8 CFU/ml with fresh broth and an inoculum of 10^5 CFU per spot was applied with an inoculating apparatus (MIC 2000 Dynatech) to agar plates containing graded dilutions of each compound (1/20–1/160). The absence of antimicrobial activity of the methanol and cremophor® mixture was checked at these level of dilutions. The MICs were defined as the highest dilution inhibiting visible growth after 18 h at 35–37 °C for bacteria and after 48 h for fungi.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2005.04.040.

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