

Monoterpene Aldehydes from *Bupleurum gibraltarium*

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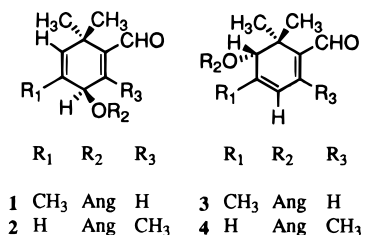
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Two new monoterpene aldehydes—3-formyl-2,2,6-trimethyl-3,5-cyclohexadienyl angelate (**3**) and 3-formyl-2,2,4-trimethyl-3,5-cyclohexadienyl angelate (**4**)—were identified from the hexane extract of *Bupleurum gibraltarium*. Their structures were elucidated by spectroscopic analysis.

As a part of our research on medicinal and aromatic plants of southern Spain and northern Morocco, we carried out a study of *Bupleurum gibraltarium* Lam. (Umbelliferae), a species whose distribution is restricted to these countries,^{1,2} where it is used in folk medicine.³ Its essential oil has been demonstrated to have marked antiinflammatory activity.^{4–6}

In a previous paper about the phytochemistry of this plant, the isolation of the monoterpene aldehydes **1** and **2** was reported.⁷ This paper describes the identification of two new natural products (**3** and **4**) from its hexane extract.



Results and Discussion

In the hexane extract of the leaves of *B. gibraltarium* Lam. the following well-known aromatic compounds and terpenoids were identified: *d*-limonene, sabinene, *p*-cymene, *m*-cymene, α -cubene, longicyclene, α -copaene, β -bourbonene, β -elemene, valencene, β -caryophyllene, α -humulene, germacrene D, γ -muurolene, γ -cadinene, *cis*-calamenene, δ -cadinene, γ -calcorene, terpineol-4, β -elemol, nerolidol, β -caryophyllene epoxide, humulene epoxide II, α -eudesmol, guaialol, 2,3,4-trimethyl benzaldehyde, 2,3,6-trimethylbenzaldehyde, **1** and **2**. In addition, we have isolated and identified two new natural products (**3** and **4**). The structures of the known compounds were determined by comparison of their physical and spectroscopic features with those reported in the literature⁸ or with those of authentic samples.

The new terpenes (**3** and **4**) were isomers of **1** and **2**. Their MS had the M⁺ at *m/z* 248, the basic peak at *m/z* 83, and other fragmentations characteristic of formyl trimethyl cyclohexadienyl esters.⁹ Compound **3** was an oil, and its IR spectrum showed absorption bands of

ester carbonyl (1713 cm⁻¹) and $\alpha\beta,\gamma\delta$ -conjugated aldehyde (2714 and 1677 cm⁻¹). The ¹H NMR spectrum (Table 1) showed signals of an angeloyl group and two doublets at 6.71 and 6.16 ppm corresponding to the olefinic protons situated in β and γ with respect to the aldehyde function. The presence of this function was confirmed by a signal at 9.42 ppm, whose chemical shift was similar to the corresponding proton in **1**.⁷ Furthermore, a signal at δ 5.35 is attributed to the proton geminal with the angelate ester. The ¹³C NMR spectrum assigned with the help of HETCOR correlation is in agreement with the structure of 3-formyl-2,2,6-trimethyl-3,5-cyclohexadienyl angelate (**3**).

The change in the position of one of the methyl groups of the compound **4** with regard to **3**, toward the 4 position, was in agreement with their chemical shift at δ 2.21 in the ¹H NMR. On the other hand, the olefinic protons H-3 and H-2 appear now at δ 6.03 and 6.23 dd, respectively, the former coupled with H-1 (δ 5.11), which holds the angeloyloxy group. Many NOE difference experiments (Table 1) corroborated the structure proposed for compound **4** as 3-formyl-2,2,4-trimethyl-3,5-cyclohexadienyl angelate.

In relation with the presence of 2,3,4- and 2,3,6-trimethylbenzaldehyde in the hexane extract, when the extraction of the aerial parts of *B. gibraltarium* with ethyl ether was carried out for 10 minutes at room temperature, the ¹H NMR spectrum of the freshly prepared extract, in acid-free deuterated, did not reveal the presence of aromatic protons. This suggests that the trimethylbenzaldehydes isolated may be artifacts of the manipulation processes. When TFA was added to a solution of terpenes **1** and **3** in the NMR tube, 2,3,4-trimethylbenzaldehyde was immediately detected. The same process with **2** and **4** afforded 2,3,6-trimethylbenzaldehyde.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using CHCl₃ as solvent. UV spectra were recorded on a Spectronic 2000 UV/vis Bausch & Lomb spectrometer, and IR spectra, on a 983G Perkin-Elmer spectrometer. NMR spectra were recorded on Bruker AMX 300, Bruker ARX 400, or Bruker AMX 500

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Table 1. NMR Spectral Data of Terpenes **3** and **4** in CDCl₃

position	3			4	
	¹³ C	¹ H (mult) (Hz)	¹³ C	¹ H (mult) (Hz)	NOE
1	77.75	5.35 s	75.75	5.11 d (4.5)	H-6, Me-2a
2	37.39		37.15		H-1, H-5
3	143.79		145.39		H-6, Me-4
4	142.60	6.71 d (5.7)	137.39		
5	121.40	6.16 d (5.7)	131.90	6.03 d (9.5)	
6	142.46		132.11	6.23 d (9.5, 4.5)	
Me-6 ^a	21.38	1.96 s	17.68	2.21 s	H-5, CHO
CHO	192.64	9.42 s	191.80	10.18 s	Me-4, Me-2b
Me-2a	23.24	1.20 s	23.85	1.20 s	H-1, CHO
Me-2b	19.12	1.30 s	19.85	1.29 s	CHO
1'	167.78		167.62		
2'	127.73		127.86		
3'	138.50	6.11 qq (7.1, 1.5)	138.46	6.05 qq (7.1, 1.5)	H-5', H-4'
4'	15.85	1.96 dq (7.1, 1.5)	15.86	1.95 dq (7.1, 1.5)	
5'	20.67	1.83 dq (1.5, 1.5)	20.67	1.86 dq (1.5, 1.5)	

^a Me-4 in **4**.

spectrometers [chemical shifts (δ) are given in ppm relative to internal Me₄Si (=0) and J values in Hertz]. MS were determined on a 5972 Hewlett-Packard mass spectrometer using an ionizing voltage of 70 eV (EIMS). Gas chromatography (GC) analysis was run on a 5890A Hewlett-Packard gas chromatograph.

Plant Material. *B. gibraltarium* Lam. was collected in the Sierra de Quentar (Granada, Spain) in May of 1995. The plant material was identified by Professor F. Valle, Department of Botany, University of Granada. A voucher specimen is available for inspection at the herbarium of the Faculty of Sciences of the University of Granada.

Extraction and Isolation. The plant material (0.98 kg) was extracted with hexane. The hexane extract was column chromatographed over Si gel, eluting with mixtures of hexane–ether of increasing polarity, giving fractions A₁–A₆. Successive chromatographic separations of fraction A₁, 1.5 g, eluted with hexane–ether (99:1), led to isolation of *d*-limonene (153 mg), *cis*-calamene (149 mg), δ -cadinene (93 mg), γ -calcorene (29 mg), a mixture of γ -muurolene and γ -cadinene (30 mg), β -caryophyllene (34 mg), germacrene D (110 mg), and α -humulene (32 mg). Fraction A₂, eluted with hexane–ether (99:1), was rechromatographed over Si gel (hexane–ether 99:1) to give terpineol-4 (42 mg); 2,3,6-trimethylbenzaldehyde (24 mg); nerolidol (18 mg); and 2,3,4-trimethylbenzaldehyde (12 mg). Fraction A₃, eluted with hexane–ether (99:1), contained a mixture of β -caryophyllene epoxide and humulene epoxide II (23 mg). Fraction A₄, eluted with hexane–ether (95:5), was rechromatographed over Si gel (hexane–ether 95:5) to give 3-formyl-4,4,6-trimethyl-2,5-cyclohexadienyl angelate (**1**) (74 mg) and 3-formyl-2,4,4-trimethyl-2,5-cyclohexadienyl angelate (**2**) (22 mg). Fraction A₅, eluted with hexane–ether (95:5), gave 3-formyl-2,2,4-trimethyl-3,5-cyclohexadienyl angelate (**3**) (106 mg) and 3-formyl-2,2,6-trimethyl-2,4-cyclohexadienyl angelate (**4**) (66 mg). Finally, fraction A₆, eluted with hexane–ether (95:5), gave β -elemol (28 mg) and a mixture of α -eudesmol, guaiol, and β -elemol (40 mg).

A sample of fraction A₁ was analyzed by GC/MS, and besides the terpenes obtained by rechromatography, sabinene, *p*-cymene, *m*-cymene, α -cubenene, longicyclene, α -copaene, β -bourbonene, β -elemene, and valencene were identified.

3-formyl-2,2,6-trimethyl-3,5-cyclohexadienyl angelate (3): yellow oil; $[\alpha]_D^{26} +196.6^\circ$ (c 1.00, CHCl₃), UV (MeOH) λ_{max} (log ϵ) 216.4 (3.5) nm; IR (film) ν_{max} 3352, 2976, 2930, 2872, 2714, 1713, 1677, 1646, 1573, 1455, 1380, 1357, 1228, 1184, 1136, 1042, 961, 834, and 761 cm⁻¹; EIMS m/z [M]⁺ 248 (4), 233(4), 219 (4), 166 (6), 147(4), 133 (5), 121 (14), 119 (7), 105 (17), 91 (9), 84 (6), 83 (100), 79 (7), 77 (9), 55 (36), 53 (5), 43 (4) and 41 (4); NMR data, see Table 1.

3-formyl-2,2,4-trimethyl-3,5-cyclohexadienyl angelate (4): yellow oil; $[\alpha]_D^{27} +194.2^\circ$ (c 1.00, CHCl₃). UV (MeOH) λ_{max} (log ϵ) 216.8 (3.6) nm; IR (film) ν_{max} 3357, 2975, 2933, 2882, 2793, 1752, 1716, 1647, 1570, 1456, 1380, 1360, 1357, 1230, 1150, 1085, 1042, 996, 830, and 756 cm⁻¹; EIMS m/z 248 [M]⁺ (2), 233 (15), 219 (5), 166 (8), 147 (3), 133 (5), 121 (12), 105 (14), 91 (9), 84 (6), 83 (100), 79 (61), 77 (8), 65 (3), 55 (36), 53 (5), 51 (3), and 43 (3); NMR data, (See Table 1).

Aromatization of 1–4 with TFA. Concentrated TFA (0.025 mL) was added to a 0.04-M solution of terpenes **1–4** in deuterated CHCl₃, in the NMR tube.

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