Chemical Compositions of the Essential Oils of the Aerial Parts of *Chamaemelum mixtum* (L.) Alloni

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Ⓢ *Supporting Information*

ABSTRACT: The chemical compositions of the aerial parts essential oils of *Chamaemelum mixtum* (L.) Alloni from Corsica and Sardinia were investigated employing gas chromatography and gas chromatography—mass spectrometry (GC-MS). The structure of (Z)-heptadeca-9,16-dien-7-one, a natural compound not previously described, was elucidated by GC-MS (electron impact and chemical ionization) and one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy. The variation in *C. mixtum* essential oil was studied, and statistical analysis showed the clustering of oil samples into three groups according to the amount of oxygenated compounds; these groups correlated to the harvest area. The strong biological activity of the oxygenated fraction (minimum inhibitory concentration of <0.1 mg/mL) of the Corsican oil against *Candida albicans, Citrobacter frendii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes,* and *Staphyllococcus aureus* can be attributed to the presence of irregular monoterpene alcohols and (Z)-heptadeca-9,16-dien-7-one.

KEYWORDS: *Chamaemelum mixtum* (L.) Alloni essential oil, chemical variability, (Z)-heptadeca-9,16-dien-7-one, irregular oxygenated monoterpes, GC-MS, NMR, antibacterial activity

INTRODUCTION

The *Chamaemelum* genus, like the *Anthemis* and *Matricaria* genera, belongs to the Asteraceae family and Anthemideae tribe. The genus has only two species: *Chamaemelum mixtum* (syn. *Ormenis mixta, Anthemis mixta, Cladanthus mixtus*), usually known as Moroccan chamomile, and *Chamaemelum fuscatum* (syn. *Ormenis fuscata, Anthemis fuscata*).[1,2] The extracts of these species have been studied, and some new sesquiterpene lactones and coumarins have been identified.[3-6]

C. mixtum is an annual species growing wild in the coastal zones of western Europe and the Mediterranean. Flowers comprise yellow capitula and white bracts, and the branched stem is erect and smooth and grows to a height of 15−60 cm. The long and narrow leaves are bipinnate or tripinnate.[1] In Morocco, *C. mixtum* is used to make a decoction to treat fever and gastric diseases. The plant is well-known and cultivated for the extraction of an essential oil from its aerial parts, which is sold and used in aromatherapy as an aphrodisiac, antibacterial, and anxiolytic.[7] To our knowledge, three studies have investigated the chemical composition of *C. mixtum* essential oils from Morocco,[8-10] in which santolina alcohol (27.9−32.0%), α-pinene (3.6−15.0%), germacrene D (3.3−10.2%), yomogi alcohol (2.8%−4.5%), and (E)-β-farnesene (2.5−4.5%) have been identified as the main components. Furthermore, the Moroccan oil of *C. mixtum* studied by Satrani et al. showed strong in vitro activity against bacteria such as *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* as well as fungi such as *Penicillium parasiticus, Aspergillus niger,* and *Trametes pini.*

The present work investigates the chemical compositions of the essential oils of the aerial parts of *C. mixtum* from Corsica and Sardinia and reports the isolation and structure of a previously undescribed natural product. In addition, the intraspecies variations of essential oils from 10 Corsican and three Sardinian sample locations were characterized by statistical analysis. Principal component analysis and cluster analysis were performed to determine the correlation between the chemical composition and possible environmental factors associated with differences in essential oils. Finally, the biological activity of *C. mixtum* essential oil from Corsica was evaluated against bacteria and yeast involved in foodborne and nosocomial infectious illnesses.

EXPERIMENTAL PROCEDURES

Plant Material and Isolation of the Essential Oil. Aerial parts of *C. mixtum* (L.) Alloni were collected in full bloom (May and June 2008) at 10 stations in Corsica [La Marana (C1, 42° 39′ 14″ N; 9° 27′ 10″ E), Embouchure Golo (C2, 42° 31′ 18″ N; 9° 32′ 3″ E), Arinella (C3, 42° 39′ 47″ N; 9° 26′ 54″ E), Quercionu (C4, 41° 56′ 46″ N; 9° 24′ 41″ E), Calvi (C5, 42° 33′ 19″ N; 8° 45′ 58″ E), Ostriconi (C6, 42° 39′ 36 ″ N; 9° 3′ 34 ″ E), Lama (C7, 42° 34′ 35″ N; 9° 9′ 6″ E), Ajaccio "Iles Sanguinaires" (C8, 41° 54′ 37″ N; 8° 38′ 50″ E), Diane (C9, 42° 7′ 10″ N; 9° 32′ 44″ E), and Folleli (C10, 42° 31′ 5″ N; 9° 31′ 59″ E)] and at 3 stations in Sardinia [Praxis (S1, 39° 7′ 0″ N; 9° 31′ 12″ E), La Caletta (S2, 40° 35′ 36″ N; 9° 45′ 21″ E), and Oristano (S3, 39° 48′ 54″ N; 8° 33′ 0″ E)]. A voucher specimen was deposited in the herbarium of University of Corsica, Corte, France. The fresh plant material was hydrodistilled (5 h) using a Clevenger-type apparatus according to the method recommended in the *European Pharmacopoeia*,[11] and the essential oil yield was 0.022−0.070%.

Chemicals. Standard compounds, solvents, and reagents were purchased from Sigma-Aldrich, except sabinene, purchased from

Extrasynthese. For the measurement of response factors (RFs), the chemicals used were neo-*allo*-ocimene, α-pinene, β-pinene, γ-terpinene, limonene, β-caryophyllene, α-humulene, aromadendrene, nerol, lavandulol, (*E*)-hex-3-en-1-ol, cedrol, globulol, pentyl acetate, lavandulyl acetate, *trans*-myrtenyl acetate, cedryl acetate, artemisia ketone, camphor, jasmone, isoborneol methyl ether, carvacrol methyl ether, caryophyllene oxide, (*E*)-2-hexenal, (*E,E*)-2,4-decadienal, and (*E*)-2-decenal.

General Experimental Procedures. *GC-FID Conditions.* Analyses were carried out using a Perkin-Elmer Clarus 600 GC apparatus (Walthon, MA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m × 0.22 mm, film thickness = 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The oven temperature was programmed from 60 to 230 °C at 2 °C min^{-1} and held isothermal at 230 °C for 30 min. Helium was employed as carrier gas (1 mL min^{-1}). The injector and detector temperatures were maintained at 280 °C, and samples were injected (0.1 μL of pure oil) in the split mode (1:80). Retention indices (RI) of compounds were determined relative to the retention times of a series of *n*-alkanes (C5−C30) by linear interpolation equation[12] with the aid of software from Perkin-Elmer (TotalChrom navigator).

Quantification of Essential Oil Components. The quantification of essential oil components was carried out using peak area normalization including response factors (RFs) with internal standard according to the methodology reported by Costa et al.[13] and modified as follows. RFs of 20 standard compounds grouped into seven chemical groups (monoterpene hydrocarbons, sesquiterpene hydrocarbons, alcohols, ketones, aldehydes, esters, others) were measured by GC (Table 2). RFs and calibration curves were determined by diluting each standard in hexane, at five concentrations, containing tridecane (final concentration = 0.7 g/100 g) as internal standard. To calculate the RF of a compound for which a standard is not available, with another one, it is indispensable that the two compounds have the same raw formula. Analysis of each standard was performed in triplicate. For quantification of essential oil components, tridecane (0.2 g/100 g) was added as internal standard in essential oil. The correction factors (averages of response factors from standards) of each chemical group were calculated and used to determine the essential oil component concentrations (g/100 g) according to their chemical group.

GC-MS-(EI) Conditions. Analyses were performed using a Perkin-Elmer Turbo mass spectrometer (quadrupole analyzer) coupled to a Perkin-Elmer Autosystem XL, equipped with two fused-silica capillary columns, Rtx-1 and Rtx-Wax. Other GC conditions were the same as described above. Ion source temperature was 150 °C, and energy ionization was 70 eV; electron ionization mass spectra were acquired with a mass range of 35−350 Da during a scan time 1 s. Oil injected volume was 0.1 μL, and fraction injected volume was 0.2 μL.

GC-MS-(CI) Conditions. PCI mass spectra with methane were recorded on the same apparatus equipped with an Rtx-Wax column and specific ionization chemical source. Other GC conditions were the same as described above. Injection volume was 0.2 μL of pure oil. The ionizing gas was methane or ammonia. Ion source temperature was 150 °C, and source pressure was 0.2 mbar. Energy ionization was 70 eV. MS (CI) were acquired over the mass range of 60−350 Da during a scan time 1 s.

NMR Analysis Conditions. The structure elucidation of **76** and its corresponding alcohol were carried out by ^{1}H and ^{13}C NMR (see the Supporting Information), DEPT, and 2D-NMR (HMBC, HSQC, COSY, and NOESY). Spectra were measured in deuterated chloroform using a Bruker Avance 400 Fourier Transform spectrometer (Wissembourg, France) operating at 100.13 MHz for ^{13}C NMR and at 400.52 MHz for ^{1}H NMR and equipped with a 5 mm probe. All shifts were referred to the internal standard tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width, 4 s (flip angle, 45°); acquisition time, 2.7 s for 128K data table with a spectral width of 25000 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/point. The number of accumulated scans was 3000−5000 for each sample depending of the amount of product.

The ^{1}H NMR spectra were recorded with the following parameters: flip angle, 30°; acquisition time, 2.56 s for 32000 data table with a spectral width of 7000 Hz (17.5 ppm). 2D-NMR sequences were recorded using Bruker microprograms.

Isolation of (Z)-Heptadeca-9,16-dien-7-one, 76. Essential oil sample C3 (8.6 g) was submitted to column chromatography on silica gel (ICN 200−500 μm, 150 g), and three fractions, FA (2630 mg), FB (1010 mg), and FC (4870 mg), were eluted successively with gradients of (v/v) pentane/diethyl ether 100:0, 90:10, and 0:100, respectively. Nine hundred and twenty milligrams of FB (**76**: 73.2%) was first submitted to LiAlH$_4$ reduction to obtain the corresponding alcohol. Successive column chromatography on silica gel (ICN 63−200 μm, 50 g) with gradient pentane/diethyl ether 90:10 (v/v) allowed concentration of (*Z*)-heptadeca-9,16-dien-7-ol (97.5%, 83 mg in FB1). **76** was obtained close to purity (97.5%, 30 mg) using pyridium chlorochromate oxidation followed by purification on CC-silica gel (ICN 63−200 μm, 5 g). Each step of the isolation process has been controlled by GC-FID, CPG-SM-(IE), and ^{13}C NMR.

Reduction of Fraction FB. Fraction FB (920 mg) was dissolved in dry diethyl ether (40 mL) and carefully added to a suspension of aluminum lithium hydride (250 mg) in dry diethyl ether (60 mL) at 0 °C. The mixture was stirred at room temperature and then refluxed for 3 h. The reaction mixture was hydrolyzed by the addition of 15% sodium hydroxide solution (1 mL) and cold water. The organic layer was separated, washed with water to neutrality, dried over sodium sulfate, and concentrated under vacuum. The mixture (850 mg) contained (*Z*)-heptadeca-9,16-dien-7-ol (76.4%) as major component.

Oxidation of Fraction FB1. Sixty milligrams of fraction FB1 ((*Z*)-heptadeca-9,16-dien-7-ol, 97.5%) was dissolved in 2 mL of CHCl$_3$ and added to a suspension of pyridinium chlorochromate (40 mg). The mixture was first stirred at 0 °C for 3 h and then at room temperature for 3 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure to give **76** with an 80.4% yield.

Identification of Compounds. *Identification of Essential Oil Constituents.* Identification of individual components in essential oil or in CC fractions was based on a methodology involving integrated techniques, such as GC retention indices, GC-MS (EI and CI), and NMR. Identifications were based on the comparison of their mass spectral pattern and RI with a laboratory-made database "Arômes" built from authentic compounds and with those of pure compounds registered in commercial libraries[14−16] and literature data. Finally, it was confirmed that all compounds identified in CC fractions were also present in all oil samples by comparison of retention indices (RIA, RIP) and EI-MS with those of the total oil.

Identification of (Z)-Heptadeca-9,16-dien-7-ol: RI$_{Rtx-1}$, 1862; RI$_{Rtx-wax}$ 2282; ^{1}H NMR (400.1 MHz, CDCl$_3$), δ 0.88 (3H, t, *J* = 6.5 Hz, H-1), 1.28 (2H, m, H-2), 1.28 (2H, m, H-3), 1.30 (2H, m, H-5), 1.35 (2H, m, H-4), 1.35 (2H, m, H-12), 1.35 (2H, m, H-13), 1.35 (2H, m, H-14), 1.45 (2H, m, H-6), 1.7 (H, s, OH) 2.03 (2H, m, H-15), 2.05 (2H, m, H-11), 2.20 (2H, t, *J* = 7.2 Hz, H-8), 3.65 (H, quin, *J* = 5.8 Hz, H-7), 4.94 (1H, ddt, *J* = 10.5 Hz, *J* = 2.1 Hz, *J* = 1.5 Hz, H-17b), 5.01 (1H, ddt, *J* = 17.1 Hz, *J* = 2.1 Hz, *J* = 1.5 Hz, H-17a), 5.4 (1H, ddt, *J* = 11.0 Hz, *J* = 6.8 Hz, *J* = 1.5 Hz, H-9), 5.55 (1H, ddt, *J* = 11.0 Hz, *J* = 7.5 HZ, *J* = 1.4 Hz, H-10), 5.4 (1H, ddt, *J* = 17.1 Hz, *J* = 10.2 Hz, *J* = 6.7 Hz, H-16); ^{13}C NMR (100.1 MHz, CDCl$_3$), δ 14.09 (3H, C-1), 22.64 (2H, C-2), 25.73 (2H, C-5), 27.38 (2H, C-11), 28.81 (2H, C-4), 28.81 (2H, C-14), 29.37* (2H, C-13), 29.53* (2H, C-12), 31.86 (2H, C-3), 33.76 (2H, C-15), 35.36 (2H, C-8), 36.87 (2H, C-6), 71.54 (H, C-7), 114.23 (2H, C-17), 125.24 (H, C-9), 134.60 (H, C-10), 139,09 (H, C-16); MS (EI, 70 eV), *m/z* (%) 55 (100), 41 (45), 43 (44), 68 (44), 81 (44), 67 (33), 97 (33), 82 (25), 96 (21), 110 (19), 138 (9).

Identification of (Z)-Heptadeca-9,16-dien-7-one, 76: RI$_{Rtx-1}$, 1827; RI$_{Rtx-wax}$ 2194; ^{1}H NMR (400.1 MHz, CDCl$_3$) and ^{13}C NMR (100.1 MHz, CDCl$_3$), see Table 1; MS (E, 70 eV), *m/z* (%) = 113 (100), 85 (40), 95 (10), 114 (10), 122 (8), 81 (5), 88 (4), 153 (3), 135 (3), 250 (2).

Statistical Analysis. The composition data matrix of the 13 samples was analyzed using principal component analysis (PCA) and hierarchical ascending classification (HCA)[17] with the aid of XLSTAT

Table 1. NMR Data of (Z)-Heptadeca-9,16-dien-7-one, 76

no.	^{13}C NMR[a]	^1H NMR[b]	HMBC[c]	COSY
1	14.05 (CH_3)	0.89 (t, $J_{1,2}$ = 6.5)	2, 3	2
2	22.53 (CH_2)	1.27 (m)	1, 3, 4	1
3	31.63 (CH_2)	1.27 (m)	1, 2, 4, 5	4
4	28.77 (CH_2)	1.35 (m)	6, 5, 3, 2	3, 5
5	23.81 (CH_2)	1.56 (tt, $J_{5,4}$ = 6.6, $J_{5,6}$ = 6.6)	7, 6, 4, 3	6, 4
6	42.39 (CH_2)	2.41 (t, $J_{6,5}$ = 6.6)	7, 8, 5, 4	7, 5
7	209.29 (C)		6, 8, 9	
8	41.68 (CH_2)	3.13 (d, $J_{8,9}$ = 6.2)	7, 10, 9, 6, 5	6, 9, 10
9	121.06 (CH)	5.54 (dtt, $J_{9,10}$ = 11.0, $J_{9,8}$ = 6.2, $J_{9,11}$ = 1.5)	8,11	8, 11
10	133.55 (CH)	5.58 (dtt, $J_{10,9}$ = 11.0, $J_{10,11}$ = 7.5, $J_{10,8}$ = 1.4)	11, 8, 12	8, 11
11	27.48 (CH_2)	2.02 (m)		
12	29.22 (CH_2)	1.35 (m)		
13	28.93* (CH_2)[d]	1.35 (m)		
14	28.81* (CH_2)	1.35 (m)	13, 12, 16, 15	
15	33.75 (CH_2)	2.03 (m)	17a, 17b, 16, 14, 13	17a, 17b, 16, 14
16	139.01 (CH)	5.80 (ddt, $J_{16,17a}$ = 17.1, $J_{16,17b}$ = 10.2, $J_{16,15}$ = 6.7)	15, 14	17a, 17b, 15
17	114.29 (CH_2)	a 4.95 (ddt, $J_{17a,16}$ = 17.1, $J_{17a,17b}$ = 2.2, $J_{17a,15}$ = 1.5)	15	16
		b 4.85 (ddt, $J_{17b,16}$ = 10.5, $J_{17b,17a}$ = 2.1, $J_{17b,15}$ = 1.2)		16

[a] δ_C, multiplicity given by DEPT is in parentheses. [b] δ_H, multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; coupling constants (apparent splitting) are reported as numerical values in hertz. [c] Signal correlating with ^1H resonance. [d] *: interchangeable values.

software (version 2009.4.06), on the basis of the components that accounted for more than 1.5 g/100 g of the total oil. PCA was made with Pearson matrix, and HCA was made with Euclidian matrix and Ward aggregation.

Antibacterial Activity. *Oil Fractionation.* Five grams of C3 sample essential oil was successively fractioned by column chromatography on silica gel (ICN 200−500 μm, 80 g) with pentane and diethyl ether to give a hydrocarbon fraction (HF) and an oxygenated fraction (OF), respectively. Both HF and OF fractions were analyzed by GC and GC-MS.

Microbial Strains. The oil was tested against eight bacteria: three Gram-positive bacteria, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*, and five Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter frendii*, *Enteroccocus faecalis*, and *Klebsiella pneumoniae*. All bacteria were isolated from the medical devices (catheters and vesicle probes) of the service surgery at the CHU of Tlemcen, and their identification was achieved with API 20 test strips (Biomerieux). They were also tested against one strain of *Candida albicans* (Ca 444) obtained from the Pasteur Institute of Algeria (IPA). All of the strains were grown on Mueller−Hinton agar (MHA) for the bacteria and Saboureaud dextrose agar (SDA) with chloramphenicol for yeasts.

Antimicrobial Screening. Antimicrobial activities are measured using the paper disk diffusion.[18] The agar plate containing the

appropriate medium was spread with the inoculums containing 10^8 CFU mL^{-1}. The filter paper disks (6 mm in diameter) were impregnated with 3 μL of the oil and then placed onto agar plates. In addition, reference disks without any oil and antibiotics (gentamicin, 15 μg; amphothericin B 100 μg) were used for comparison. After incubation at 37 ± 1 °C for 18−24 h for bacteria and at 30 ± 1 °C for 24−48 h for yeast, the diameters of inhibition zones were measured and are reported in Table 3.

Minimal inhibitory concentrations (MICs) were determined using the dilution agar method.[19] Serial dilutions of the oil were carried out in Mueller−Hinton agar medium. Appropriate volumes of every dilution were added to this medium to obtain the required concentration range and a final concentration of Tween 80 at 10% (v/v). Two controls were included in this test. Each dish contained a sterile solution of Tween 80 and the culture medium, respectively. After incubation at 37 ± 1 °C for 18−24 h for bacteria and at 30 ± 1 °C for 48 h for yeast, the MICs were defined as the lowest concentration of the oil (Table 3) at which the microorganism did not demonstrate visible growth. Antibiotics used were the same as described above.

■ RESULTS AND DISCUSSION

Oil Components. The combined use of CC, GC-RI, GC-MS (EI and CI), and NMR analyses of Corsican and Sardinian *C. mixtum* essential oils identified the same 78 components in each sample, which accounted for 89.9−93.5 g/100 g of the total amount (Table 2). The 78 components were distributed as 11 hydrocarbon monoterpenes (1.3−8.6 g/100 g), 20 oxygenated monoterpenes (21.0−57.2 g/100 g), 15 hydrocarbon sesquiterpenes (6.1−50.7 g/100 g), 13 oxygenated sesquiterpenes (3.2−17.4 g/100 g), and 19 nonterpenic compounds (5.5−16.4 g/100 g). Corsican and Sardinian *C. mixtum* oils were qualitatively similar but differed in terms of the relative concentrations of their major components. The main components were three irregular monoterpenes well-known in Asteraceae essential oils, namely, santolina alcohol, **18** (12.5−46.2 g/100 g), yomogi alcohol, **13** (0.7−16.2 g/100 g), and artemisia alcohol, **22** (1.3−13.2 g/100 g); three hydrocarbon sesquiterpenes, namely, germacrene D, **54** (0.5−28.6 g/100 g), (E,E)-α-farnesene, **57** (0.2−15.6 g/100 g), and (E)-β-farnesene, **52** (1.9−11.3 g/100 g); and compound **76**. All components have been identified by comparing their GC retention indices and mass spectra with those of our laboratory-produced "Arômes" library, except for 12 compounds that have been identified from commercial libraries.[14,15,20]

Compound **76**, which amounted to 4.4−12.7 g/100 g of *C. mixtum* essential oils, remained unidentified. To complete the identification, we investigated the Corsican *C. mixtum* sample oil (C3) in which **76** accounted for 10.7 g/100 g. The isolation of **76** required combining successive chromatography columns and chemical transformations to change the polarity and improve the resolution of CC separation (see Materials and Methods). The unknown compound was obtained close to purity (**76**, 97.5 %) by employing this analytical procedure, and its structure was elucidated by GC-MS-(EI), GC-MS-(CI), and 1D- and 2D-NMR.

EI mass spectra of **76** exhibited a base peak at m/z 43 and two other peaks at m/z 113 and 85. Because a weak signal at m/z 250, which could be attributed to the molecular ion, was observed in the EI mass spectra, the molecular mass 250 was deduced by positive and negative chemical ionization mass spectrometry (PCI- and NCI-MS). Pseudomolecular ions [M − H]$^-$ at m/z 249 and [M + H]$^+$ at m/z 251 were observed in NCI-NH$_3$ and PCI-CH$_4$ mass spectra, respectively. Addition-

Table 2. Chemical Compositions of Corsican and Sardinian C. mixtum Essential Oils and Hydrocarbon and Oxygenated CC Fractions Obtained from Corsican Sample C3

no.	components	LRI[b]	RI$_A$[c]	RI$_p$[d]	Corsican samples										Sardinian samples			C3 fractions[a]		identification[e]
					C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	S1	S2	S3	HF	OF	
1	heptane	700*	700	700	f	t		t	t	t	t	t	t	t	t	t	t	t		RI, MS, Std
2	3-methylbutanol	718*	716	1178	0.1	t	0.1	t	0.1	t	t	t	t	t	0.2	0.2	0.1		0.1	RI, MS, Std
3	2-methylbutanol	724*	717	1170	0.1	0.1	t	t	t	t	t	t	t	t	t	0.1	t		0.1	RI, MS, Std
4	hexanal	770*	770	1055	0.1	0.1	0.1	t	t	0.1	0.1	0.1	t	t	0.2	0.1	0.1		0.2	RI, MS, Std
5	3-methylpentanol	825*	824	1299	0.1	0.2	0.2	t	t	0.1	t	0.1	t	t	0.2	0.3	0.2		0.2	RI, MS, Std
6	santolinatriene[g]	909	905	1018	t	0.7	0.1	0.4	0.1	0.3	0.1	0.3	1	0.2	t	0.4	t	0.3		RI, MS
7	α-pinene	936	933	992	1.3	1.7	1.5	3.9	1.2	1.9	2.0	2.1	2.4	1.0	0.6	3.5	0.6	5.6		RI, MS, Std
8	camphene	942	940	1035	0.1	0.2	0.2	0.3	0.1	0.5	0.1	0.3	0.2	t	t	0.2	t	0.7		RI, MS, Std
9	6-methylhept-5-en-2-one	972	969	1311	0.2	0.2	0.2	0.1	0.1	0.1	0.1	t	0.1	0.1	0.3	0.6	0.4		0.4	RI, MS, Std
10	sabinene	973	970	1095	0.1	0.1	0.1	0.1	0.1	0.1	t	t	0.1	t	t	0.1	t	0.3		RI, MS, Std
11	β-pinene	978	976	1078	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	t	0.5	0.1	0.1	0.3		RI, MS, Std
12	myrcene	987	987	1130	0.4	0.2	0.2	3.6	0.1	0.1	t	t	0.7	0.2	0.1	0.9	0.2	0.9		RI, MS, Std
13	yomogi alcohol[g]	991	990	1387	16.2	14.3	15.8	3.6	0.7	6.4	1.2	4.9	4.0	2.3	2.2	2.2	2.9		19.9	RI, MS
14	m-cymene	1013	1011	1268	0.1	0.2	0.2	0	0.1	0.1	t	t	0.2	t	t	0.1	t	0.6		RI, MS, Std
15	p-cymene	1015	1012	1267	0.1	0.1	0.1	0	t	0.1	t	t	t	t	0.1	0.2	0.2	0.3		RI, MS, Std
16	1,8-cineole	1024	1020	1209	1.6	2.1	2.1	0.6	0.1	0.1	0.1	0.1	0.3	0.5	0.4	0.5	0.3		2.7	RI, MS, Std
17	limonene	1025	1021	1243	0.1	0.2	0.2	0.1	t	t	t	t	0.2	0.1	t	0.1	t	0.5		RI, MS, Std
18	santolina alcohol[g]	1029	1026	1395	12.5	15.6	13.9	26.2	17.1	21.3	21.2	21.9	22.9	15.4	43.3	46.2	39.8		21.5	RI, MS
19	(E)-oct-2-enal	1034	1032	1253	0.1	0.1	0.1	t	t	0.1	t	t	t	t	0.1	0.1	t		0.2	RI, MS, Std
20	artemisia ketone[g]	1044	1047	1352	0.1	t	0.1	t	t	t	t	t	t	0.2	t	t	0.1		0.1	RI, MS
21	γ-terpinene	1051	1046	1243	t	0.1	0.1	0.1	t	0.1	0.1	t	t	t	0.1	0.1	0.1	0.2		RI, MS, Std
22	artemisia alcohol[g]	1073	1072	1479	12.0	12.7	13.2	2.1	1.3	2.4	1.9	2.1	1.4	2.8	4.1	4.7	3.4		20.1	RI, MS
23	6-methylhepta-3,5-dien-2-one	1083*	1077	1084	0.1	0.1	0.1	t	t	0.1	t	t	t	t	0.1	0.1	0.1		0.1	RI, MS, Std
24	terpinolene	1082	1078	1280	0.1	t	t	t	t	t	t	t	t	t	0.3	0.1	0.1	t		RI, MS, Std
25	linalool	1086	1081	1544	0.1	0.5	0.5	0.2	0.4	0.1	0.1	t	3.0	0.8	0.1	0.5	0.3		0.7	RI, MS, Std
26	nonanal	1083*	1083	1394	0.1	t	t	0.2	t	0.1	0.3	0.2	0.2	0.2	0.4	0.1	0.3		t	RI, MS, Std
27	hotrienol[g]	1083	1083	1575	0.3	t	t	t	t	0.1	t	0.2	t	t	t	t	t		t	RI, MS
28	2-methylbutyl isovalerate	1094*	1098	1274	0.1	0.1	0.1	0.3	0.2	0.1	0.2	0.2	0.2	t	0.2	0.2	0.1		0.2	RI, MS, Std
29	trans-pinocarveol	1126	1120	1650	0.2	0.2	t	0.2	0.2	0.3	0.2	0.3	0.1	0.3	0.5	0.3	0.1		0.2	RI, MS, Std
30	(E)-non-2-enal	1137	1132	1568	0.1	0.1	t	t	t	t	t	t	t	t	0.1	t	t		t	RI, MS, Std
31	pinocarvone	1139	1136	1558	0.1	0.1	0.1	0.1	0.2	t	0.2	0.2	0.1	0.1	0.3	0.2	1.3		0.1	RI, MS
32	borneol	1150	1148	1698	0.3	0.3	0.3	0.2	0.2	0.4	0.3	0.3	0.3	0.2	0.7	0.7	1.2		0.4	RI, MS, Std
33	terpinen-4-ol	1164	1160	1600	0.2	0.3	0.3	0.1	0.1	t	0.1	t	0.1	t	0.2	0.2	0.2		0.5	RI, MS, Std
34	myrtenal	1172	1172	1628	t	0.1	0.1	0.1	0.1	0.1	0.1	t	0.1	1	0.2	0.1	0.1		0.2	RI, MS, Std
35	α-terpineol	1176	1173	1178	t	0.1	t	0.1	t	t	0.1	t	t	t	0.1	t	t		0.1	RI, MS, Std
36	bornyl formate	1199	1212	1557	0.4	0.6	0.6	t	0.1	0.3	0.2	0.1	0.1	t	0.2	0.1	0.1		0.8	RI, MS
37	thymol methyl oxide[g]	1215	1214	1589	0.1	0.1	0.1	0.1	0.1	t	0.1	t	t	t	0.6	0.1	0.1		0.1	RI, MS
38	geraniol	1235	1237	1731	0.1	0.1	t	t	t	t	t	t	0.1	t	0.1	t	t		0.1	RI, MS, Std
39	(E)-dec-2-enal	1240	1248	1652	t	0.1	t	0.2	0.2	0.1	0.1	0.1	t	t	0.2	0.1	0.1		0.1	RI, MS, Std
40	bornyl acetate	1270	1267	1540	0.4	0.5	0.5	0.3	t	0.9	0.2	0.2	0.6	t	0.7	0.7	0.1		0.6	RI, MS, Std
41	undecan-6-ol[g]	1283	1282	1676	0.1	t	t	t	t	t	t	t	t	t	0.1	0.1	0.1		t	RI, MS
42	(E,E)-deca-2,4-dienal	1291	1290	1820	0.1	t	0.1	t	t	t	t	t	t	t	0.1	0.1	0.2		0.2	RI, MS, Std

Table 2. continued

no.	components	LRI[b]	RI_A[c]	RI_P[d]	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	S1	S2	S3	HF	OF	identification[e]
					Corsican samples										Sardinian samples			C3 fractions[a]		
43	(Z)-hex-3-enyl tiglate	1297*	1305	1641	t	t	t	t	0.1	0.1	0.1	0.1	0.2	t	0.1	t	0.1		t	RJ, MS, Std
44	myrtenyl acetate	1304	1306	1680	t	t	t	t	0.1	0.2	t	t	t	t	t	t	t		t	RJ, MS, Std
45	trans-2-undecenal	1342*	1361	1757	0.1	0.1	0.1	0.1	0.5	0.3	t	0.7	0.2	0.2	t	t	0.3		0.2	RJ, MS, Std
46	geranyl acetate	1362	1363	1752	0.1	0.1	0.3	0.2	t	0.4	0.3	t	0.3	0.2	0.9	0.4	0.3		0.4	RJ, MS, Std
47	(Z)-jasmone	1371	1373	1909	0.1	0.1	0.1	0.3	0.2	t	0.2	0.1	0.5	1.1	0.4	0.1	0.5		0.1	RJ, MS
48	α-ylangene[g]	1376	1375	1476	t	t	t	t	0.1	0.3	0.4	0.1	t	t	0.1	0.1	0.2	0.5		RJ, MS
49	β-elemene[g]	1389	1384	1589	0.1	0.1	0.1	t	0.2	t	0.3	0.3	0.3	0.3	0.2	0.1	0.1	0.7		RJ, MS
50	β-caryophyllene	1420	1416	1547	1.2	1.1	1.1	1.0	1.9	1.5	2.3	1.9	0.9	1.1	0.1	0.2	0.7	4.5		RJ, MS, Std
51	β-ylangene[g]	1420	1420	1562	t	0.1	0.1	0.2	0.3	t	0.4	t	0.1	0.3	0.1	0.1	0.1	0.3		RJ, MS
52	(E)-β-farnesene	1446	1445	1626	6.8	5.2	5.3	3.4	7.5	3.0	5.8	5.0	1.9	11.3	3.0	4.3	5.6	20.3		RJ, MS, Std
53	γ-muurolene[g]	1474	1471	1681	0.1	0.2	0.1	0.4	0.1	t	t	0.1	0.4	0.4	0.1	0.8	0.2	0.4		RJ, MS
54	germacrene D[g]	1479	1478	1661	9.0	6.0	7.0	25.2	24.1	21.7	28.6	20.2	20.5	13.3	0.6	0.5	1.2	29.4		RJ, MS
55	zingiberene[g]	1489	1489	1717	0.4	1	0.3	0.2	0.2	0.2	1.4	0.3	0.5	0.7	0.2	0.1	0.8	1.0		RJ, MS
56	bicyclogermacrene[g]	1494	1494	1727	0.2	0.4	0.2	0.7	1.1	0.9	0.7	0.8	0.5	0.5	0.2	0.1	0.5	0.7		RJ, MS
58	α-muurolene[g]	1496	1496	1719	t	t	t	t	t	t	t	t	t	t	0.1	0.2	0.2	0.2		RJ, MS
57	(E,E)-α-farnesene[g]	1498	1498	1705	6.6	6.5	6.5	3.8	13.3	7.8	4.2	15.6	3.4	6.1	0.2	0.2	2.2	26.0		RJ, MS
59	γ-cadinene[g]	1507	1507	1752	0.2	0.2	0.1	0.3	0.3	0.2	0.5	0.2	0.4	0.5	0.1	0.2	0.4	0.3		RJ, MS
60	δ-cadinene[g]	1520	1513	1749	0.6	0.5	0.6	1.2	1.3	0.9	2	1.7	1.4	1.2	0.2	0.2	0.2	2.9		RJ, MS
61	cadina-1,4-diene[g]	1523	1523	1763	t	t	t	t	0.1	t	t	t	t	t	0.5	t	0.1	t		RJ, MS
62	α-cadinene[g]	1534	1536	1745	t	0.3	0.3	0.1	0.2	0.3	0.2	0.1	0.1	0.7	0.4	0.3	0.6	1.0		RJ, MS
63	(E)-nerolidol	1553	1549	2037	0.4	0.3	0.3	0.4	0.7	0.1	0.1	t	0.4	0.9	1.4	0.9	0.5		0.4	RJ, MS, Std
64	1,5-epoxysalvial-4-ene[g]	1571	1568	1870	0.3	0.3	0.3	0.2	0.4	0.3	0.2	0.2	0.4	0.6	0.9	0.3	0.5		0.4	RJ, MS
65	spathulenol[g]	1572	1570	2119	0.4	0.2	0.2	t	0.2	0.2	0.2	0.2	0.9	1.1	1.7	0.5	0.6		0.2	RJ, MS
66	caryophyllene oxide	1578	1570	1980	0.8	0.4	0.6	0.2	0.4	0.5	0.2	0.4	0.3	1.0	2.2	0.7	1.7		0.8	RJ, MS, Std
67	globulol[g]	1589	1589	2074	0.4	0.9	0.7	0.3	0.1	0.3	0.2	0.1	0.2	1.7	1.0	0.4	2.4		0.8	RJ, MS, Std
68	copaborneol[g]	1595	1592	2159	0.3	0.5	0.4	0.1	0.3	0.4	0.1	0.1	0.9	1.7	0.7	0.3	0.2		0.5	RJ, MS
69	ledol[g]	1600	1599	2029	0.4	0.5	0.4	0.1	0.3	0.4	1	0.3	0.2	0.8	0.2	0.3	0.2		0.5	RJ, MS
70	aromadendrene oxide	1613*	1617	2002	0.2	0.2	0.2	0.1	0.2	0.3	0.5	0.2	0.2	0.5	0.3	0.3	0.7		0.2	RJ, MS, Std
71	12-epi cedrol[g]	1620	1620	2163	0.3	0.7	0.7	0.1	0.3	0.2	0.3	0.2	0.2	2.0	1.3	0.6	1.3		0.9	RJ, MS
72	τ-muurolol[g]	1633	1634	2143	0.8	0.2	0.2	0.6	1.2	1.1	1.8	1.5	2.4	0.7	1.7	0.7	1.5		0.3	RJ, MS
73	τ-cadinol[g]	1633	1638	2163	0.1	0.8	0.8	1.0	1.9	2.0	0.4	2.5	3.1	2.6	0.8	0.3	0.4		1.1	RJ, MS
74	α-cadinol[g]	1643	1645	2227	1.1	0.1	0.1	t	0.1	0.3	3.2	0.1	0.3	0.5	0.9	0.3	0.6		0.1	RJ, MS
75	germacra-4,5,10-trien-1α-ol[g]	1676	1680	2298	0.8	0.7	0.6	0.1	0.8	0.4	0.7	0.4	0.6	3.3	0.8	0.4	1.2		0.8	RJ, MS
76	(Z)-heptadeca-9,16-dien-7-one	1827		2194	9.9	10.5	10.7	7.9	9.2	9.1	4.4	5.7	11.5	10.2	12.5	12.3	12.7		16.9	RJ, MS, NMR
77	tridecane	2300[f]	2300	2300	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.4	0.2	0.1	0.7	0.3		RJ, MS, Std
78	pentacosane	2500*	2500	2500	t	0.2	0.2	t	0.2	0.1	0.2	0.1	0.1	0.6	0.2	0.1	0.9	0.6		RJ, MS, Std
	total identified				90.1	90.7	90.5	91.7	91.0	90.0	89.9	92.9	91.7	91.9	90.5	90.9	93.5	98.8	94.5	
	hydrocarbon compounds				27.7	25.5	24.8	45.4	52.9	40.3	49.4	49.3	35.6	38.9	8.2	13.4	16.0	98.8		
	oxygenated compounds				62.4	65.2	65.7	46.3	38.1	49.7	40.5	43.6	56.1	53.0	82.3	77.5	77.5		94.5	
	hydrocarbon monoterpenes				2.4	3.6	2.8	8.6	1.8	3.3	2.3	2.8	5.0	1.5	1.7	5.8	1.3	9.7		
	oxygenated monoterpenes				44.8	47.8	48.4	34.5	21.0	33.1	26.4	30.4	33.8	24.9	55.0	57.2	50.9		71.5	

Table 2. continued

no.	components	LRI[b]	RI_A[c]	RI_P[d]	Corsican samples										Sardinian samples			C3 fractions[a]		identification[e]
					C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	S1	S2	S3	HF	OF	
	oxygenated irregular monoterpenes				40.7	40.6	39.9	31.9	16.1	30.1	24.3	28.9	25.3	15.5	49.6	53.1	34.1		61.5	
	hydrocarbon sesquiterpenes				25.2	21.6	21.7	36.7	50.7	33.8	46.8	46.3	30.4	36.4	6.1	7.4	13.1	88.2		
	oxygenated sesquiterpenes				6.3	5.8	5.5	3.2	6.9	6.5	8.9	6.2	10.1	17.4	12.5	6.0	11.8		7.0	
	nonterpenic hydrocarbon compounds				0.1	0.3	0.3	0.1	0.4	0.2	0.3	0.2	0.2	1.0	0.4	0.2	1.6	0.9		
	nonterpenic oxygenated compounds				11.3	11.6	11.8	8.6	10.2	10.1	5.2	7.0	12.2	10.7	14.8	14.3	14.8		16.0	
	yields (w/w vs fresh material)				0.20	0.19	0.22	0.70	0.54	0.51	0.55	0.60	0.59	0.63	0.24	0.26	0.45			

[a]Hydrocarbon (HF) and oxygenated (OF) fractions from *C. mixtum* (C3 sample) from Corsica, obtained by column chromatography, respectively. Order of elution is given on apolar column (Rtx-1). [b]Literature retention indices on apolar column reported from ref 14 except those with "*", which are reported from ref 20. [c]Retention indices on Rtx-1 (apolar) column. [d]Retention indices on Rtx-Wax (polar) column. g/100 g of individual components on Rtx-1 except those with same RI_Aj g/100 g given on Rtx-Wax. [e]Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those of authentic sample or from the literature; Std, by co-injection of an authentic sample, NMR, by Nuclear Magnetic Resonance (1D and 2D experiments). [f]t = trace (<0.05 g/100g). For the major components (e.g., >1%), the coefficients of variation were always <6.0%. [g]Compounds tentatively identified according to its mass spectrum and by comparison of its retention indices with those of the commercial libraries (14-16) and literature.

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ally, PCI-NH$_3$ mass spectra showed a signal adduct [M + NH$_4$]$^+$ at m/z 268.

The ^{13}C NMR spectra of **76** exhibited 17 carbon signals, which were assigned according to the distortionless enhancement by polarization transfer (DEPT) spectra to 1 methyl carbon at δ_C 14.05 ppm, 11 methylene carbons with chemical shifts between δ_C 42.69 and 22.53 ppm, 1 exomethylene carbon at δ_C 114.29 ppm, 3 ethylenic methine carbons at δ_C 139.01, 133.55, and 121.06 ppm, and a quaternary carbon at δ_C 209.29 ppm characteristic of a carbonyl group (Figure 1). The
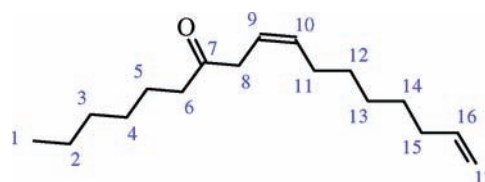

Figure 1. Structure of (Z)-heptadeca-9,16-dien-7-one, **76**.

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molecular formula C$_{17}$H$_{30}$O deduced from the DEPT spectra requires three degrees of unsaturation, accounted for by two olefinic carbons and one carbonyl carbon. Here, we assigned **76** to a linear carbonyl compound.

The ^{1}H NMR spectrum of **76** displayed one triplet (δ_H 0.89) methyl signal, two shielded methine signals at δ_H 3.13 (2H, d) and 2.41 (2H, t), and several multiplets between δ_H 1.27 and 2.41. Additionally, the ^{1}H NMR spectrum showed two downfield signals at δ_H 5.54 (1H, dtt) and 5.58 (1H, dtt) and three deshielded ddt signals (δ_H 4.85, 4.95, 5.80) involved in the three-proton AMX system, suggesting the presence of a terminal allylic moiety. The carbonyl group was assigned to a ketone because no aldehydic proton was observed in the downfield region of the ^{1}H spectra. Heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC), and correlation spectroscopy (COSY) experiments confirmed the structure of a diunsaturated linear ketone (Figure 1).

The position of the ketone was established from joint information from EI-MS and two-dimensional NMR spectra. Characteristic mass fragments at m/z 113 [C$_7$H$_{13}$O]$^+$ and 85 [C$_6$H$_{13}$]$^+$, corresponding to the α-keto-β-allylic break (C-7−C-8) and charge-induced break (C-6−C-7), respectively, indicated that the ketone group was at the C-7 position. Additionally, the strongly shielded nature of H-6 and H-8, respectively, δ_H 2.41 (2H, t) and 3.13 (2H, m), and the correlations in the HMBC experiment between C-7/H-6 and C-7/H-8 confirmed that the ketone group was at the C-7 position. The nonconjugated nature of the carbonyl group was directly confirmed by observation of cross-peaks in the HMBC spectrum between signals at δ_C 209.29 (s, C-7) and δ_H 3.13 (2H, d, H-8), which had COSY correlations with the two ethylenic protons of the double bond δ_H 5.54 (1H, dtt, H-9) and 5.58 (1H, dtt, H-10). Furthermore, the strong downfield proton signal at δ_H 3.13 (2H, d, C-8) confirmed the localization between the ketone and allylic double bond. HSQC, HMBC, and COSY correlations confirmed the localization of the $\Delta^{9,10}$ double bond on the linear chain. The relative configuration of the $\Delta^{9,10}$ double bond was established by analysis of ^{1}H coupling patterns and the steric effect observed in ^{13}C NMR spectra. The medium coupling constant ($^3J_{\text{H-9,H-10}}$ = 11.0 Hz) was in agreement with a Z configuration. Moreover, the Z configuration of the $\Delta^{9,10}$ double bond was confirmed by the

shielded methylene signals at δ_C 41.68 (C-8) and 27.48 (C-11), which are characteristic of a γ steric effect between two carbons relative to compounds with E configuration ($\delta_{C8} \sim$ 45 ppm, $\delta_{C11} \sim$ 32 ppm, respectively).[21]

Finally, we unambiguously identified **76** as (Z)-heptadeca-9,16-dien-7-one, a previously undescribed natural product that probably originates biosynthetically along the fatty acid pathway or, although less likely, from carotenoid decay.

Essential Oil Description and Variability. Aerial parts of 13 *C. mixtum* samples from Corsica and Sardinia were hydrodistilled to afford essential oils with moderate yields: 0.020–0.070% of fresh material. The oils were investigated to obtain better insight into the chemical composition and variability. The standardized essential oil matrix was statistically analyzed employing hierarchical ascending classification and PCA. The dendrogram and plot established using the first two axes, which accounted for 30.44 and 19.70% of the total variance, suggest the existence of three clusters (Figures 2 and
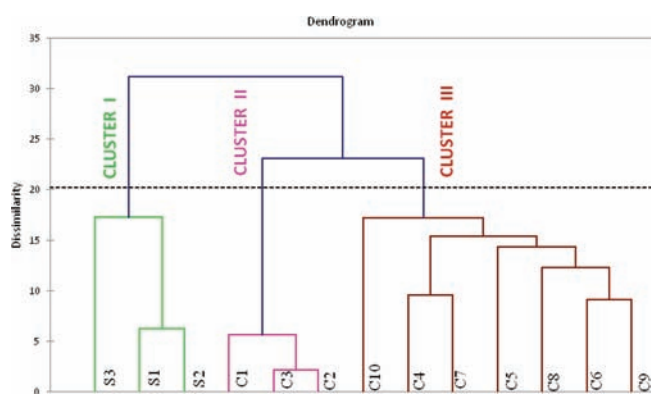

Figure 2. Dendrogram from hierarchical ascending classification of *C. mixtum* sample oils from Corsica (C1–10) and Sardinia (S1–3).

3). Figure 3 shows the distribution of the discriminating volatile compounds: santolina alcohol, **18**; yomogi alcohol, **13**; artemisia alcohol, **22**; (E,E)-α-farnesene, **57**; (E)-β-farnesene, **52**; (Z)-heptadeca-9,16-dien-7-one, **76**; and germacrene D, **54**,
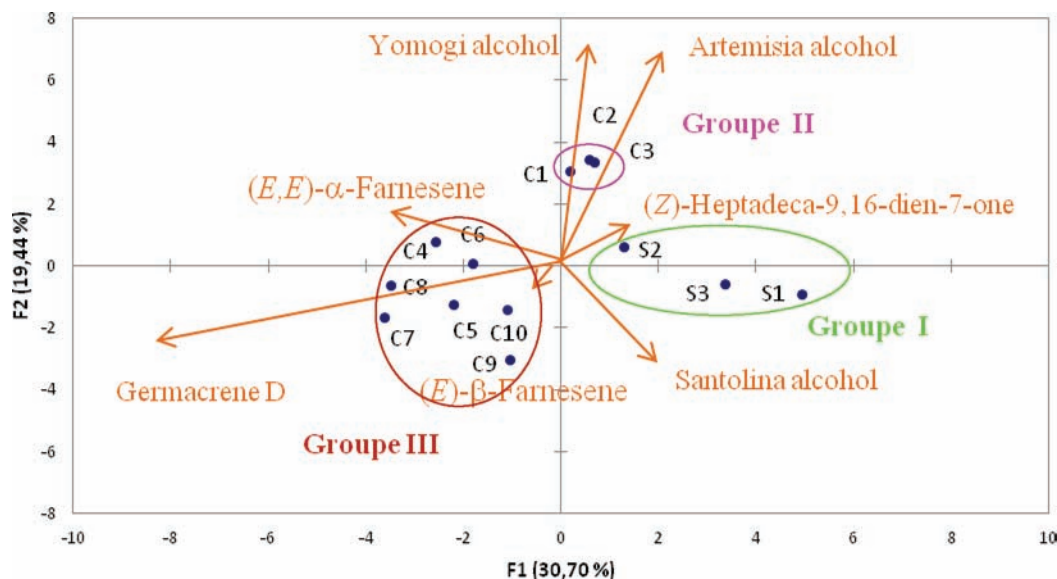
as well as the distribution of oil samples. The F1 axis was negatively correlated with hydrocarbon sesquiterpenes **57**, **52**, and **54** and positively correlated with oxygenated compounds **13**, **18**, and **22** as well as **76**.

Cluster I included three Sardinian sample oils dominated by oxygenated compounds (77.6–82.4 g/100 g) including oxygenated irregular monoterpenes (34.1–53.1 g/100 g). The amount of hydrocarbon compounds was low (<16.0 g/100 g). The main components were santolina alcohol, **18** (39.8–46.2 g/100 g), and heptadeca-9,16-dien-7-one, **76** (12.3–12.7 g/100 g). Cluster II included three Corsican sample oils characterized by lower concentrations of oxygenated compounds (62.4–65.7 g/100 g) than for cluster I and higher amounts of hydrocarbon compounds (24.8–27.7 g/100 g). The major compounds were yomogi alcohol, **13** (14.3–16.2 g/100 g); santolina alcohol, **18** (12.5–15.6 g/100 g); artemisia alcohol, **22** (12.0–13.2 g/100 g)' and (Z)-heptadeca-9,16-dien-7-one, **76** (9.9–10.7 g/100 g); therefore, the amount of irregular oxygenated monoterpenes was quite similar (39.6–40.7 g/100 g) to that for cluster I. Finally, cluster III included the seven other Corsican oil samples. These oil samples were characterized by higher amounts of hydrocarbon sesquiterpenes (30.4–50.7 g/100 g) and lower amounts of irregular oxygenated monoterpenes (15.5–31.9 g/100 g) than for clusters I and II. The main components were santolina alcohol, **18** (15.4–26.2 g/100 g); germacrene D, **54** (13.3–28.6 g/100 g); (E,E)-α-farnesene, **57** (3.4–15.6 g/100 g); and (E)-β-farnesene, **52** (1.9–11.3 g/100 g). We note that *C. mixtum* samples from cluster III had higher essential oil yields (0.51–0.70%) than those from the two other clusters (0.19–0.45%).

The chemical variability between Corsican and Sardinian *C. mixtum* sample oils reported here can be explained by environmental factors. The variability was linked to the harvest area. Whereas samples from cluster I were clearly correlated to the Sardinian harvest area, Corsican samples from cluster II were localized in a weaker area near Bastia (in the northeast of the island), and the samples of cluster III were distributed in more disparate areas of Corsica. It is not surprising to find such results owing to the well-known difference of soils between these two distinct geological areas.[2]

Figure 3. Principal component analysis of *C. mixtum* sample oils from Corsica (C1–10) and Sardinia (S1–3).

Table 3. Antibacterial Activities of *C. mixtum* Essential Oil

microorganisms	DD[a] (mm)				MIC[b] (μg/mL)			
	EO[c]	HF[d]	OF[e]	ATB[f]	EO	HF	OF	ATB
Gram-positive bacteria								
Bacillus cereus	11	10	**15**	18	900	825	**88.5**	4
Staphylococcus aureus	**30**	17	**25**	20	**90**	**82.5**	**85.8**	2
Listeria monocytogenes	**15**	11	**15**	18	**90**	825	**85.8**	2
Gram-negative bacteria								
Escherichia coli	**25**	18	**20**	20	**90**	858	**85.8**	4
Pseudomonas aeruginosa	10	9	11	11	1000	1000	1000	4
Citrobacter frendii	**30**	20	**24**	19	**90**	825	**85.8**	4
Enteroccocus faecalis	**25**	16	**31**	18	**90**	825	**85.8**	4
Klebsiella pneumoniae	**30**	19	**32**	20	**90**	825	**85.8**	4
yeast								
Candida albicans	12	11	**20**	18	900	8255	**85.8**	1

[a]Agar disk diffusion method. Diameter of inhibition zone (mm) including well diameter of 6 mm. [b]Minimum inhibitory concentration. [c]Essential oil sample C3 from Arinella (Corsica). [d]Hydrocarbon fraction obtained by CC from C3 oil sample (7: 5.6 g/100 g, 54: 20.3 g/100 g, 54: 29.4 g/100 g and 57: 26.0 g/100 g). [e]Oxygenated fraction obtained by CC from C3 oil sample (13: 19.9 g/100 g, 18: 21.5 g/100 g, 22: 20.1 g/100 g and 76: 16.9 g/100 g). [f]Antibiotic, gentamicine (15 μg) for bacteria, amphotericin B (5 μg) for yeast.

Compared with previously described Moroccan *C. mixtum* essential oil,[8−10] the chemical compositions of Corsican and Sardinian essential oil samples included a newly described linear ketone, (Z)-heptadeca-9,16-dien-7-one, **76** (4.4−12.7 g/100 g), as a major compound. It is notable that the chemical compositions of the essential oil samples from Corsica (clusters II and III), particularly essential oil samples from cluster II, had original chemical compositions, with high levels of yomogi alcohol, **13** (14.3−16.2 g/100 g), and artemisia alcohol, **22** (12.0−13.2 g/100 g).

Antibacterial Activities. The antimicrobial activities of *C. mixtum* essential oils from Morocco are already known.[8] As mentioned in the previous section, samples belonging to cluster II from Corsica are the most unique of Moroccan essential oils previously described,[8−10] and we therefore investigated the antimicrobial activities of sample C3. This sample and both hydrocarbon and oxygenated fractions obtained by CC were tested in vitro, employing the agar diffusion method and the minimum inhibitory concentration (MIC) in liquid phase, against eight bacteria (five Gram-negative and three Gram-positive) and one yeast (Table 3). The results obtained employing disk diffusion indicated that the essential oil and CC fractions had a strong antimicrobial effect against *S. aureus*, *E. coli*, *C. freundii*, *E. faecalis*, *L. monocytogenes*, and *K. pneumoniae* (15−30 mm). The oxygenated fraction also demonstrated good activity against *C. albicans* yeast (20 mm). MIC results did not show significant activity for the hydrocarbon fraction, except against *S. aureus*. However, the MIC results for the essential oil showed strong activity against *S. aureus*, *E. coli*, *C. freundii*, *E. faecalis*, *L. monocytogenes*, and *K. pneumoniae* (90.0 μg/mL). Better MIC results were obtained for the oxygenated fraction, which was active against all bacteria (85.8 μg/mL) except *P. aeruginosa*. The oxygenated fraction had good activity against *C. albicans* yeast with a lower MIC (85.8 μg/mL).

The antibacterial and antifungal potential of the oxygenated CC fraction can be explained by its main components: santolina alcohol, **18** (21.5 g/100 g); artemisia alcohol, **22** (20.1 g/100 g); yomogi alcohol, **13** (19.9 g/100 g); and (Z)-heptadeca-9,16-dien-7-one, **76** (16.9 g/100 g). As seen in previous work, irregular monoterpene alcohols have antibacterial activity,[22,23] and linear unsaturated ketones have good antibacterial and antifungal activities.[24]

Essential oil from Corsica had the same activity against *S. aureus* and *E. coli* as Moroccan[8] *C. mixtum* oil, but this is the first report of *C. mixtum* biological activity against *C. albicans*, *C. freundii*, *E. faecalis*, *K. pneumoniae*, and *L. monocytogenes*. These results confirm the high potential of *C. mixtum* oil as a natural antimicrobial to prevent the growth of bacteria and yeast in food and medicines.

ASSOCIATED CONTENT

S Supporting Information

(Z)-Heptadeca-9,16-dien-7-one ^{13}C and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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