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ESSENTIAL OIL COMPOSITION OF *CISTUS ALBIDUS* LEAVES

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Key Word Index—*Cistus albidus*; Cistaceae; rockrose; essential oil; sesquiterpenes; α-zingiberene.

Abstract—The essential oils of *Cistus albidus* leaves growing wild on calcareous and siliceous soils in Provence (South of France) were analysed by GC and GC-MS. The major component, for both oils, is α-zingiberene (116.5–45.2 $\mu g\ \mu l^{-1}$), and differences in essential oil composition between the *Cistus* populations from the two soil types, as well as the chemotaxonomic aspect of α-zingiberene accumulation, are discussed. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Cistus* (Cistaceae) comprises 10 species in France [1]. These shrubs, known as rockrose, are indigenous to the Mediterranean area [2]. They colonize degraded areas, so they have an increased distribution as a result of human disturbance [3]. Numerous chemical studies have been conducted on *Cistus* species [4–6], particularly their essential oils [7–9]. They produce a number of compounds which are used in the fragrance industry [10, 11], and the essential oils of some species have cytotoxic and antibacterial properties [10, 12, 13].

Among those plants, *Cistus albidus* L. is one of the most common in Provence (France). It is a semi-deciduous shrub up to one meter high [14, 15] with malacophyllous and resinous leaves. The leaf surface shows a very dense cover of hair which gives it a pale green, cottony, character. It is the only *Cistus* species with pilous leaves. Few studies have been made on chemical composition of *Cistus albidus*. Some addressed flavonoids [16, 17], while others described cuticular or epicuticular waxes [18, 24]. No mention of the existence of *Cistus albidus* essential oil has been reported in literature, probably because of its pilous leaves and its discrete odour, even though its leaves contain trichomes as reported by Henning [24] and Gülz [25].

During a study of the secondary metabolism of *Cistus albidus*, we have discovered that it is possible to obtain essential oils from this species and now report their composition. Because *Cistus albidus* may grow on both siliceous and calcareous soils, we have studied the differences of the essential oil composition between the plants from these two substrates.

RESULTS AND DISCUSSION

The *Cistus albidus* essential oils have a light yellow colour and a disagreeable odour. The mean yield (*w/w* in dry weight) in essential oils (0.13% ± 0.06) is higher than that obtained by Gülz [7] for other *Cistus* species (0.02%–0.08% in fresh weight). Some studies made on *Cistus ladaniferus* (Garzino, S., personal communication) allow us to assert that this difference can not be attributed to the species or to the use of dry or fresh plant material. Indeed, the essential oils yield of *Cistus ladaniferus* (0.17% ± 0.06 in fresh weight) is even higher than that obtained for *Cistus albidus*. These yields may perhaps be explained by different climatic conditions. Provence has a Mediterranean climate characterized by a low amount of precipitation during summer, the hottest period, and spring. Moreover, the sunlight intensity is also very high. Numerous studies have demonstrated the influence of climatic factors on secondary metabolite production in plants [27, 28]. Particularly, the water supply and the light regime have a strong influence on the essential oil yield [29, 30]. Differences in oil yield between plants growing under Mediterranean climates and those growing under lower light intensity or rainier climates have already been reported for the genus *Mentha* [30]. The oil yield varies according to the study site and also to the sampling month (Table 1). *Cistus*

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Table 1. Essential oil yield (*w*/*w*) according to the study site and to the sampling month

	November	December	January
Ca1	0.037	0.084	0.042
Ca2	0.103	0.123	0.069
Ca3	0.113	0.128	0.108
*Ca mean	0.084 ± 0.043	0.112 ± 0.024	0.073 ± 0.020
Si1	0.182	0.166	0.098
Si2	0.129	0.218	0.124
Si3	0.243	0.165	0.150
**Si mean	0.243 ± 0.057	0.165 ± 0.030	0.150 ± 0.026

*Mean of the three calcareous samplings (± S.E.).
**Mean of the three siliceous samplings (± S.E.).

plants growing on calcareous soils always afforded the smallest yields (0.09% ± 0.03 and 0.16 ± 0.06 for plants located on calcareous and siliceous soils respectively). A Wilcoxon Mann-Whitney's U test shows that these yield differences are significant ($p = 0.0023$). The variations in essential oil yield during the study period of three months are similar for the *Cistus* on calcareous soils but not for plants on siliceous substrate. In both cases, these changes are not caused by phenological events because these months correspond to a period of vegetative rest for *Cistus albidus* [14, 15]. The variations in essential oil content are likely caused by changes in environmental conditions which suggests a great influence of abiotic factors on the essential oil production by *Cistus albidus*.

The major components of the essential oil from *Cistus albidus* leaves are shown in Table 2. The compounds are arranged in order of elution. Although some could not be identified, they appear in Table 2 because they are present in high concentration. The oils are mainly constituted by sesquiterpenes. The sesquiterpene hydrocarbons are the major components followed by the sesquiterpene alcohols which are also well represented. The essential oils contain also some alkanes (docosane and pentacosane) but in very small quantities as trace components.

The comparison of the data in Table 2 demonstrates that there are no qualitative differences between the compositions of essential oils from the *Cistus albidus* from both substrates. However, a Wilcoxom-Mann Whitney's U test shows significant concentration differences for some components. The essential oils from *Cistus albidus* on calcareous soils are significantly richer in allo-aromadendrene ($p = 0.0071$), β-caryophyllene ($p = 0.0305$) and β-caryophyllene epoxide ($p = 0.0380$) than the oils from plants on siliceous soils. However, they are significantly poorer in β-bourbonene ($p = 0.0071$), unidentified compounds M ($p = 0.0071$) and U ($p = 0.0041$) and in α-cucurmene ($p = 0.0152$). The quantitative modification of the essential oil composition according to the substratum can be only the consequence of different nutrients and water availabilities according to the soil type. A different water and nutrient supply may induce changes in plant secondary metabolite production [27, 30]. The influence of substrates on the chemical composition of the essential oils has been described already for *Thymus vulgaris* [32, 33]. However, the major differences could not be due only to environmental factors. Indeed, the soil type changes the chemotype frequency. In this study, the existence of chemotypes cannot be suggested for *Cistus albidus* because leaves were harvested on 20 individuals in each study site. However, the plants growing on the two soil types may be two different ecotypes. Other studies are needed to verify if these two populations are genetically determined or if these differences are caused by influences of the soil type.

For each essential oil sample, the major component was α-zingiberene (116.5–45.2 $\mu g\ \mu l^{-1}$). Other compounds present in significant amounts were the unidentified sesquiterpene—peak H—(52.0–33.1 $\mu g\ \mu l^{-1}$), δ-cadinene (46.7–30.2 $\mu g\ \mu l^{-1}$) and α-cucurmene (40.8–22.0 $\mu g\ \mu l^{-1}$). Some identified components are common to several other *Cistus* species, namely β-caryophyllene, α-humulene, allo-aromadendrene, δ-cadinene and docosane [7–13, 36–38]. The presence of α-zingiberene as major component of *Cistus albidus* oils is all the more surprising as it has not been reported for the oils of the other *Cistus* [7–13, 36–38]. On the other hand, it is an important component of the essential oil from *Zingibere officinale* rhizomes [34, 35]. *Cistus albidus* is the only species of the genus *Cistus* whose essential oil contains α-zingiberene and of which it represents the major component. For this reason, this compound may be considered as a characteristic constituent of this species.

Some other reported compounds have not been yet described in the essential oils of other *Cistus* species. They are α-cucurmene, α-bisabolol, β-caryophyllene epoxide, α-cadinol, T-muurolol and pentacosane [7–13, 36–38]. The existence of pentacosane in *Cistus albidus* cuticular waxes and in epicuticular waxes [20, 21, 24] may explain its presence in the essential oils of this species.

It is also important to note that no monoterpene was detected in these oils even as a trace constituent. All other species of the genus *Cistus* contain some monoterpenes [7, 10–12, 37, 38, 40]. The absence of monoterpene loss during essential oil preparation has been verified by adding some known monoterpenes (α-pinene, β-pinene and *p*-cymene) to plant samples and carrying through the preparation. The absence of monoterpene may explain the very discrete odour of this species.

Therefore, the essential oils of *Cistus albidus* are very different from those of the other *Cistus*, and in agreement with Gülz [7], we conclude that the species of this genus present different essential oil chemical compositions. As these species can hybridize easily and the hybrids are hardly recognizable by morphological characters [1, 2], a classification and identi-

Table 2. Constituents detected in the essential oils of *Cistus albidus* leaves.

Peaks	R. Time	Name	Concentrations ($\mu g\ \mu l^{-1}$)*								
			Ca1	Ca2	Ca3	Si1	Si2	Si3	Mean Ca**	Mean Si***	Mean
A	16.2	β-Bourbonene	5.8±0.1	12.2±1.3	14.5±1.1	14.4±5.1	16.0±1.3	15.6±0.4	10.8±1.3	15.4±6.9	13.1±4.1
B	22.4	Unknown	3.7±0.8	4.4±0.8	4.3±0.2	4.3±1.1	4.1±0.3	4.2±0.1	4.1±0.02	4.2±2.3	4.2±0.6
C	22.9	β-Caryophyllene	36.9±2.3	39.4±3.2	19.4±2.0	21.8±5.6	19.8±1.4	18.4±5.9	31.9±10.9	20.0±5.6	26.0±9.5
D	28.5	Allo aromadendrene	32.1±10.2	30.5±6.6	34.0±4.4	20.7±11.0	19.9±8.7	23.1±8.0	32.2±6.9	21.2±9.0	26.7±9.2
E	31.2	α-Humulene	traces	traces	traces	traces	traces	traces	traces	traces	traces
F	37.2	α-Curcumene	31.2±5.1	40.8±9.7	22.0±1.8	51.4±10.6	33.6±9.0	38.4±1.5	31.3±14.9	41.1±15.3	36.2±11.1
G	39.6	α-Zingiberene	116.5±34.1	71.9±23.7	45.2±5.0	76.8±19.1	67.7±16.6	84.8±21.8	77.9±17.0	76.4±31.6	77.1±28.6
H	44.2	Unknown Sesquiterpene	51.3±5.8	52.0±5.6	33.1±2.8	45.3±5.8	42.1±5.9	44.7±4.9	45.5±9.6	44.0±21.1	44.7±7.8
I	47.1	δ-Cadinene	46.7±9.2	33.2±8.4	29.8±7.1	30.2±6.1	38.2±6.6	39.5±2.3	36.6±1.9	36.0±18.7	36.3±8.5
J	61.8	Unknown	22.3±2.6	27.1±6.3	16.5±3.9	23.5±3.0	24.7±2.4	25.5±2.9	22.0±5.4	24.6±12.4	23.3±4.7
K	62.9	Unknown	11.5±1.7	20.0±6.2	21.1±2.5	19.8±1.8	27.5±6.8	25.1±5.5	17.5±0.7	24.1±12.1	20.8±6.5
L	71.8	β-caryophyllene epoxide	14.1±6.5	15.8±3.1	15.1±0.7	9.7±1.2	13.6±3.2	11.2±1.9	15.0± 3.3	11.5±5.9	13.3±3.6
M	79.9	Unknown	17.8±0.4	23.6±6.7	14.9±2.7	23.2±2.3	24.0±3.8	22.4±0.7	18.7±4.9	23.2±11.3	21.0±4.6
N	91.8	Elemol	traces	traces	traces	traces	traces	traces	traces	traces	traces
O	95.9	Unknown	10.0±5.5	5.4±2.8	7.0±2.2	6.3±2.0	6.4±2.2	7.4±2.3	7.5±0.8	6.7±3.1	7.1±3.0
P	97.6	Unknown	12.5+4.4	9.4±2.1	8.5±2.4	9.4±1.7	11.8±0.5	11.5±0.4	10.1±0.6	10.9±5.8	10.5±2.5
Q	104.5	T-Muurolol	10.8±3.2	11.5±3.2	9.0±1.0	10.4±1.0	11.7±3.4	11.2±2.1	10.5±1.3	11.1±6.0	10.8±2.3
R	106.5	α-Cadinol	20.1±4.3	18.6±4.3	17.4±3.3	18.9±1.3	21.2±1.5	21.2±2.2	18.7±0.8	20.4±11.3	19.5±3.0
S	108.3	Docosane	traces	traces	traces	traces	traces	traces	traces	traces	traces
T	111.9	α-Bisabolol	24.8±5.6	25.1±7.3	24.0±4.4	26.7±0.6	27.7±3.5	27.7±6.5	24.6±1.4	27.4±15.1	26.0±4.6
U	117.5	Unknown	9.5±1.8	5.9±3.0	4.9±1.7	10.5±2.5	10.9±1.6	10.1±1.4	6.8±2.9	10.5±4.6	8.7±3.0
V	120.9	Unknown	7.4±1.7	7.2±3.3	7.9±0.8	5.1±2.0	9.9±3.0	9.1±2.8	7.5±1.4	8.0±4.5	7.8±2.6
W	145.6	Unknown	1.8±0.9	1.4±0.2	1.2±0.1	2.5±0.6	1.9±0.7	1.0±0.3	1.5±0.7	1.8±0.7	1.6±0.7
X	163.2	Pentacosane	traces	traces	traces	traces	traces	traces	traces	traces	traces
Y	174.6	Unknown	3.7±1.5	1.5±0.5	1.5±0.5	1.2±0.4	1.4±0.3	1.2±0.3	2.2±0.2	1.3±0.2	1.8±1.1

*Mean of the three samplings (±S.E.).
**Mean of the calcareous samplings (±S.E.).
***Mean of the siliceous samplings (±S.E.).

fication according to essential oil composition could be of great taxonomic utility.

EXPERIMENTAL

Plant material

Leaves of *Cistus albidus* L. (Cistaceae) were harvested from 20 individuals randomly chosen in six study areas in the wild. These study sites are located on both substrates. Ca1, Ca2 and Ca3 are calcareous sites near Marseille, and Si1, Si2 and Si3 are siliceous sites in the "Massif des Maures". The voucher specimens from Ca1 (95–101 to 95–120), Ca2 (95–121 to 95–140), Ca3 (95–141 to 95–160), Si1 (95–161 to 95–180), Si2 (95–181 to 95–200), Si3 (95–201 to 95–220), are deposited in the Herbarium of the Faculty of Sciences (St Jérôme), University of Marseille, France. *Cistus albidus* leaves were collected in each study area on the same days in November and December 1995 and January 1996.

Analysis of the essential oils

The dried leaves of *Cistus albidus* were subjected to steam distillation for 2.5 h. The essential oils were diluted in hexane and were analyzed by GC and GC-MS using a capillary column FFAP: 50 m × 0.32 mm × 0.52 μm. The column temperature was programmed: from 100°C (isotherm at 100°C for 30 min) to 130°C at 1°C min^{-1} (isotherm at 130°C for 20 min); from 130°C to 170°C at 1°C min^{-1} (isotherm at 170°C for 20 min) and from 170°C to 230°C at 2°C min^{-1} (isotherm at 230°C for 8 min). Injector temp. was 270°C (injection split 1/100). The carrier gas was He (flow rate: 30 ml min^{-1}) and the injection vol. for the sample was 2 μl. For the GC analysis, the detector was a flame ionization detector (air, H_2) at 270°C. *p*-cymene was used as internal standard. For the GC/MS analysis, the MS conditions were: temp. ion source: 240°C; energy: 70eV; electron current: 300 μA.

Identification of chromatographic peaks was made by comparison of their mass spectra with those of the internal reference mass spectra library [26] and by comparison of the retention time values with those of the commercial standards (Aldrich, Firminich).

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