The Composition of Terpene Hydrocarbons in the Essential Oils from Leaves of Four Cistus Species

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The hydrocarbon fractions of the essential oils from leaves of *Cistus ladanifer, C. palhinhae, C. laurifolius* and *C. monspeliensis* were analysed by GLC and GCMS techniques.

The Mediterranean region is known to be the natural habitat of the genus *Cistus L.* (Cistaceae). Only two *Cistus* species are, however, endemic to the Canary Islands, *C. symphytifolius* and *C. osheckiaefolius*. Cistus plants are important members in two typical ecosystems of this region, the evergreen Maquis and the Garigues [1–4].

The characteristic odour of these vegetation types is often determined by fragrance compounds from Cistus plants. Leaves of all Cistus species are covered with glands which secrete essential oils and resins at the leaf surface. Labdanum resin and Cistus oils are produced from these secretions. Whereas Labdanum resin was formerly an officinal drug, Cistus oils, especially those from *C. ladanifer, C. monspeliensis, C. creticus* and *C. solvifolius*, still are used in perfumery [5–7].

Cistus oils have been analysed since 1950 by several authors and nearly all monoterpenes identified [8–15]. Although it was possible to separate the different terpenes by GLC and HPLC [16], the chemical structures of most sesquiterpenes and diterpenes could not be elucidated. Thus, this paper presents a reinvestigation of the monoterpenes and analysis of the sesquiterpenes of Cistus oils using GLC techniques and GC-combined mass spectrometry. The study concentrates on those Cistus species which produce the greatest amount of resin (10.7–13.5% dry wt) [17], *i.e., C. ladanifer, C. palhinhae, C. laurifolius* and *C. monspeliensis*.

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Materials and Methods

The Cistus plants were grown from seeds and cultivated under equal conditions in the fields of the Botanical Institute of the University of Cologne. Whole leaves and stems were harvested in autumn and extracted by steam distillation for 7 h. Essential oils could be obtained by this method in amounts of 0.02–0.08% fresh weight. Maceration of plant material must be avoided because of enzymatic reactions and the occurrence of artifacts [13]. Essential oils from Cistus species consist of very complex mixtures of terpenoids. A fractionation of the oils according to the functional groups of the components was achieved on silica gel columns by elution with different eluents of increasing polarity. Hydrocarbons were eluted with pentane [12].

The chemical structures of the hydrocarbon fraction components were elucidated by GLC and GCMS techniques, comparing retention data and spectra with those from authentic samples as well as with data from the literature [18–22] and from previous work [23].

Gas chromatography

1. Hewlett Packard 5830, flame ionization detector, 12 m fused silica capillary column OV101, temp. progr.: 80–280 °C, 4°/min; inject split 1:100, injector 220 °C, detector 260 °C; carrier gas N_2, 10 psig ≙ 20 cm/s.

2. Perkin Elmer Sigma 1, flame ionization detector, 50 m fused silica capillary column SE54, temp. progr.: 4 min at 60 °C, 60–260 °C, 3°/min, hold;

Table I. Composition of the hydrocarbon fractions of the essential oils of *Cistus ladanifer*, *C. laurifolius*, *C. palhinhae* and *C. monspeliensis*. The compounds are represented quantitatively in percentage values (FID), the retention indices RI have been determined on SE54, the column labelled GC gives cochromatography data. The mass spectra were recorded at 70 eV, significant fragment ions and intensities are given.

No.	Compound identified and references	percentage [%] in the oil of				RI	GC	Mass spectrum: *m/e* (rel. intensity %)
		ladanifer	*laurifolius*	*palhinhae*	*monspeliensis*			
1	C_9H_{16}	0.20				857		124(M^+, 14), 109(100), 91(14), 81(32), 67(55)
2	tricyclene	0.81	1.23			923	a	136(M^+, 22), 121(27), 93(100), 79(29), 77(23)
3	thujene	0.18	0.05	trace	trace	927	a	136(M^+, 15), 121(19), 93(100)
4	α-pinene [8, 9, 11, 12]	43.2	25.2	trace	0.04	935	a	136(M^+, 8), 121(12), 93(100), 79(25), 77(27)
5	camphene [8, 11, 12]	12.2	23.2	trace	trace	949	a	136(M^+, 17), 121(65), 93(100), 79(56), 77(30)
6	fenchene [24]	1.20	0.23	0.14		954		unresolved GC peak in MS mode
7	sabinene [11, 12]	0.24		trace	0.04	974	a	136(M^+, 13), 121(5), 93(100), 79(30), 77(51)
8	β-pinene [8, 12]	0.87	4.15		0.02	978	a	136(M^+, 7), 121(12), 93(100), 80(14), 79(26)
9	$C_{10}H_{14}$	0.38				992		134(M^+, 39), 119(63), 91(100), 77(44)
10	myrcene [12]		0.60	trace	trace	992		136(M^+, 5), 121(9), 93(52), 69(49), 41(100)
11	$C_{10}H_{14}$	0.41	0.13			1006		134(M^+, 20), 119(43), 91(100), 77(43)
12	α-phellandrene [11, 12]					1006	a	136(M^+, 16), 93(100), 77(31)
13	Δ^3-carene			trace		1011	a	incomplete spectrum
14	α-terpinene [11, 12]	0.93	0.36			1018	a	136(M^+, 48), 121(92), 93(100), 91(42), 77(45)
15	*p*-cymene [11, 12]	0.54	0.14			1026	a	136(M^+, 30), 119(100), 103(4), 91(30)
16	limonene [11, 12]	3.56	2.88	0.19	0.04	1029	a	136(M^+, 27), 121(29), 93(50), 68(100), 67(95)
17	γ-terpinene [12]	2.54	1.06	trace	0.03	1058	a	136(M^+, 36), 121(30), 93(100), 91(66), 77(44)
18	terpinolene	0.70	0.49	0.15		1088	a	136(M^+, 66), 121(84), 93(100), 77(44)
19	undecane			0.30		1100	b	156(M^+, 2), 71(54), 57(88), 43(100)
20			0.46			1109		148(M^+, 21), 119(100), 105(7), 91(12), 77(4)
21			0.29			1124		174(3), 172(6), 137(7), 136(6), 123(19), 81(100)
22			0.46			1139		150(M^+, 30), 121(49), 93(100), 77(26)
23		0.22				1163		172(M^+, 1), 157(4), 155(11), 136(30), 95(100), 81(28)
24	dodecane			0.55		1200	b	170(M^+, 2), 71(49), 57(79), 43(100)
25		0.24				1261		192(M^+, 28), 177(14), 135(59), 122(100), 109(58)
26	$C_{13}H_{24}$		0.46			1265		180(M^+, 3), 165(3), 137(12), 123(18), 69(100)
27					0.30	1276		122(58), 107(100), 105(39), 93(31), 91(33)
28	tridecane			0.70		1300	b	184(M^+, 3), 71(66), 57(79), 43(100)
29					0.08	1314		163(21), 161(48), 71(62), 57(100), 43(84)
30		0.62				1353		204(M^+, 9), 161(67), 119(91), 105(100), 91(50), 81(37)
31		1.82				1372		204(M^+, 20), 161(45), 105(100), 94(64), 93(65)
32	ylangene		0.08		0.07	1372	a	204(M^+, 24), 161(50), 120(72), 119(76), 105(100)
33	α-copaene	1.29	0.15		0.40	1381	a	204(M^+, 12), 161(78), 119(96), 105(100)
34	sesquiterpene $C_{15}H_{24}$	0.34				1395		204(M^+, 12), 161(50), 108(100), 105(62)
36	tetradecane			0.75		1400	b	198(M^+, 2), 71(77), 57(92), 43(100)
38	α-gurjunene	0.31				1415		204(M^+, 40), 189(38), 161(80), 105(100), 91(61)
39	caryophyllene	0.47	6.39		0.22	1425	a	204(M^+, 5), 189(7), 161(19), 133(67), 120(32), 41(100)
40	sesquiterpene $C_{15}H_{24}$	0.13				1447		204(M^+, 17), 161(62), 122(75), 107(100)
41	sesquiterpene $C_{15}H_{24}$				0.48	1447		204(M^+, 8), 121(45), 108(100), 93(73), 91(75)
42	probably α-cubebene	0.33				1457		204(M^+, 17), 161(100), 119(67), 105(94)
43	humulene	0.02	0.13		0.04	1462	a	204(M^+)
44	probably acoradiene		1.68			1466		204(M^+, 2), 147(38), 121(70), 119(100), 105(87)
45	γ-gurjunene or allo-aromadendrene	2.28			0.73	1468		204(M^+, 25), 161(50), 105(72), 91(88), 41(100)

Table I. (continued)

No.	Compound identified and references	percentage [%] in the oil of				RI	GC	Mass spectrum: *m/e* (rel. intensity %)
		ladanifer	*laurifolius*	*palhinhae*	*monspeliensis*			
46	sesquiterpene $C_{15}H_{24}$	0.84				1480		204(M^+, 22), 161(100), 134(18)
47	sesquiterpene $C_{15}H_{24}$	0.44			0.16	1482		204(M^+, 17), 161(90), 119(84), 93(100), 91(96)
49	probably δ-guaiene	0.39				1494		204(M^+, 17), 107(85), 105(95), 93(100)
50	sesquiterpene $C_{15}H_{24}$	0.40				1496		204(M^+, 9), 161(89), 105(100)
51	pentadecane			0.89	0.02	1500	b	212(M^+, 2), 71(60), 57(72), 43(100)
52	probably viridiflorene	1.95				1502		204(M^+, 21), 161(48), 135(46), 107(100)
53	α-muurolene	0.74				1506		204(M^+, 12), 161(37), 105(100)
55	γ-cadinene	0.15				1522	a	204(M^+)
56	δ-cadinene	6.57			0.33	1531		204(M^+, 35), 161(100), 134(62), 119(72), 105(66)
57	sesquiterpene $C_{15}H_{24}$	0.29				1533		204(M^+)
58	probably cadina-1,4-diene	0.58				1540		204(M^+, 16), 161(45), 119(100), 105(73), 91(31)
60	hexadecane	0.03	0.11	0.82	0.10	1600	b	226(M^+, 2), 71(79), 57(88), 43(100)
62	heptadecane	0.10	0.12	1.01	0.37	1700	b	240(M^+, 2), 71(70), 87(91), 43(100)
65	octadecane	0.01	0.13	1.14	1.12	1800	b	254(M^+, 2), 57(89), 43(100)
75	nonadecane	0.42	2.08	2.63	1.37	1900	b	268(M^+, 2), 71(69), 57(87), 43(100)
76						1900		
83	diterpene $C_{20}H_{32}$	0.24			3.51	1973		272(M^+, 25), 257(33), 119(100), 187(66), 127(50)
85	diterpene $C_{20}H_{32}$				0.56	1991		
86	eicosane	0.07	0.19	1.20	0.22	2000	b	282(M^+, 2), 71(79), 57(82), 43(100)
87	diterpene $C_{20}H_{32}$	0.43			7.51	2052		272(M^+, 17), 257(55), 229(42), 213(28), 69(100)
88	diterpene $C_{20}H_{32}$	0.96			41.8	2071		272(M^+, 10), 189(100), 161(28), 119(29), 107(32)
89	heneicosane	0.96	0.33	2.63	1.21	2100	b	296(M^+, 1), 71(98), 57(98), 43(100)
91	methyl-heneicosane	0.09		0.51				
92	docosane	0.10	0.23	1.42	0.69	2200	b	310(M^+, 2), 71(67), 57(96), 43(100)
94	tricosane	1.82	0.63	33.8	3.60	2300	b	324(M^+, 2), 71(53), 57(69), 43(100)
95	methyl-tricosane	0.20	0.03	1.33		2363		
96	tetracosane	0.07	0.23	1.28	0.27	2400	b	338(M^+, 2), 71(66), 57(96), 43(100)
97	pentacosane	0.20	1.19	3.75	0.95	2500	b	352(M^+, 2), 71(55), 57(67), 43(100)
98	hexacosane	0.04	1.12	0.30	0.26	2600	b	366(M^+, 1), 71(68), 57(84), 43(100)
99	heptacosane	1.28	2.53	11.5	9.44	2700	b	380(M^+, 2), 71(69), 57(100), 43(100)
100	octacosane	0.14	0.25	0.50	0.76	2800	b	394(M^+, 1), 71(70), 57(89), 43(100)
101	nonacosane	1.22	3.01	10.2	8.62	2900	b	408(M^+, 2), 57(78), 43(100)
102	triacontane	0.02	0.03	0.20	0.94	3000	b	422(M^+, 1), 57(91), 43(100)
103	hentriacontane	0.03	0.25	0.40	1.45	3100	b	436(M^+, 1), 57(67), 43(100)

[a] Cochromatography with SE54; [b] Cochromatography with SE30.

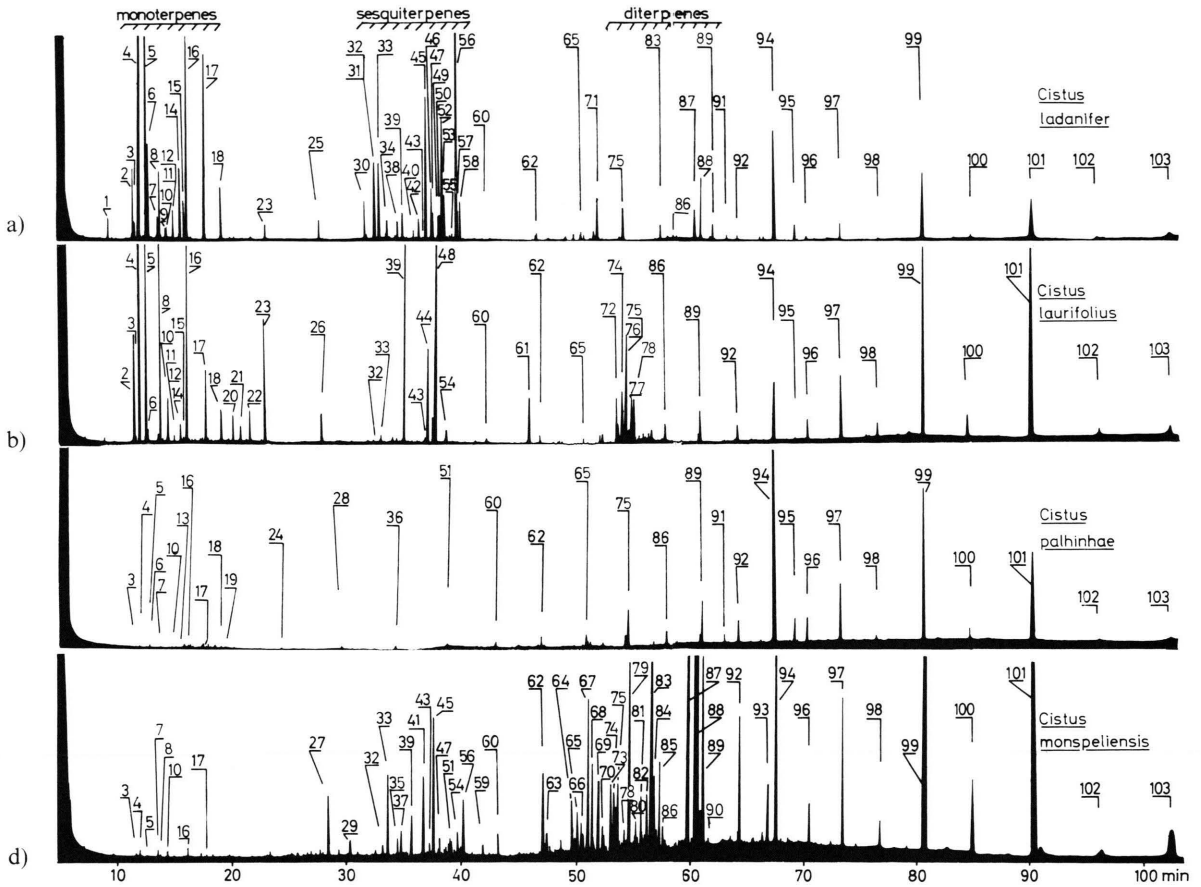


Fig. 1. Gas chromatograms of the hydrocarbon fractions of the essential oils from a) *Cistus ladanifer*, b) *C. laurifolius*, c) *C. palhinhae* and d) *C. monspeliensis*, fused silica WCOT capillary column SE54 (GC conditions given on p. 699, the signal numbers refer to Table I).

inject split 1:100, injector 220 °C, detector 260 °C; carrier gas N_2, 14 psig ≙ 22 cm/s.

3. Perkin Elmer Sigma 1, flame ionization detector, 25 m fused silica column SE30, temp. progr.: 10 min at 70 °C, 70–220 °C, 4°/min; inject split 1:100, injector 220 °C, detector 240 °C; carrier gas N_2, 14 psig ≙ 23 cm/s.

Mass spectrometry

Quadrupole GCMS combination Finnigan 3200E with Data System 6000, 25 m fused silica column SE30, direct coupling with the ion source, temp. progr.: 5 min at 70 °C, 70–240 °C, 6°/min, hold; carrier gas He, 10 psig; 70 eV EI spectra, 3 s/scan.

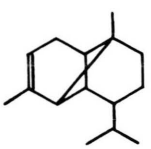
The determination of Kovats indices, RI, was carried out with the SE54 capillary column, choosing a temperature program with a linear function of the retention times t_R of *n*-alkanes C_{10} to C_{18} with the number of carbon atoms. The indices were compared with those measured with nonpolar GC phases like SE30, Apiezon L, OV1, OV101 and Sil5, and in most cases the values did not differ more than two index units.

Results and Discussion

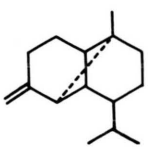
The essential oils of the four Cistus species investigated consisted of complex hydrocarbon mixtures differing widely in their composition (*c.f.*, Fig. 1). Whereas the *C. ladanifer* oil contained a great number of mono- and sesquiterpenes (about 87%, Table I), the oil of *C. laurifolius*, belonging to the same section *Laudanium*, was poorer in these terpenes in favour of increased amounts of alkane

hydrocarbons. The third member of this section which was investigated, *C. palhinhae*, showed only traces of monoterpene hydrocarbons. Its oil, with respect to hydrocarbon composition, was very similar to extracts of leaf waxes of these species. The essential oil of the fourth species investigated, *C. monspeliensis* of the *Stephanocarpus* section, was also poor in mono- and sesquiterpene hydrocarbons. It consisted, however, of a diterpene, $C_{20}H_{32}$, representing more than 40% as a main component in addition to a considerable amount of alkanes.

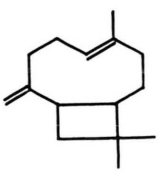
Thus, despite the close relationship of the four Cistus species investigated, the isolated oils from these species show different terpene compositions. Particularly striking is the difference between *C. ladanifer* and *C. palhinhae*, two Cistus species which can hardly be distinguished by their outward appearance and morphology.



ylangene (**32**)

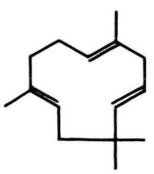


α-copaene (**33**)

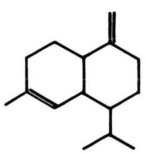


caryophyllene (**39**)

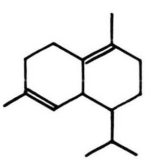
From the oil of *C. ladanifer* some monoterpene hydrocarbons were already known [8–15]; in addition we have shown the presence of thujene (**2** in Table I and Fig. 1), tricyclene (**3**) and terpinolene (**18**). Furthermore, beside *p*-cymene (**15**) two more compounds, $C_{10}H_{14}$ (**9** and **11**, mw. 134), were found. Δ^3-Carene was identified as a trace component in *C. palhinhae*.



humulene (**43**)



γ-cadinene (**55**)



δ-cadinene (**56**)

In the sesquiterpene fraction (Fig. 1) of *C. ladanifer*, δ-cadinene (**56**), with 6.57% represents the main component. In addition, the presence of ylangene (**32**), α-copaene (**33**), caryophyllene (**39**), humulene (**43**) and γ-cadinene (**55**) was indicated by cochromatography and comparison of spectra with authentic samples. Additionally, because of a good conformity of mass spectra as well as retention indices with those derived from literature, we identified α-gurjunene (**38**) and α-muurolene (**53**). Furthermore, the spectra of GC peaks **42, 45, 49, 52** and **58** resembled those of α-cubebene, γ-gurjunene or alloaromadendrene, δ-guaiene, viridiflorene and cadina-1,4-diene, respectively. (The chemical structures of the sesquiterpenes identified are given in the formula scheme).

Different was the sesquiterpene composition of the other three Cistus species: ylangene (**32**), copaene (**33**), caryophyllene (**39**) and humulene (**43**) are also found in *laurifolius* and *monspeliensis* (see formula scheme), but in different concentrations. However, the *ladanifer* main component, δ-cadinene (**56**), is not present in the other two *Ladanium* species at all. On the contrary, in the *laurifolius* oil, besides caryophyllene (**39**, 6.39%), another main substance (**48**, 5.02%) with a retention time of sesquiterpenes was detected, the structure of which we cannot say for certain.

The only member investigated from the section *Stephanocarpus*, *C. monspeliensis*, besides alkanes, almost exclusively contained diterpene hydrocarbons, $C_{20}H_{32}$, as main components (**83, 87** and **88** with 3.51, 7.51 and 41.8%, respectively, Fig. 1 and Table I).

The manifold alkanes, eluted as a homologous series from C_{11} to C_{31}, occurring in all Cistus oils, represent constituents of epicuticulary waxes of these plants. They are also codistilled with steam and thus can be found in the oil. They are also reported by other authors to be present in essential oils [25]. The large amount of these alkanes may be attributed to the long distillation period of 7 h. The *n*-alkane hydrocarbons often were accompanied by traces of corresponding methylbranched hydrocarbons, as can be seen from Fig. 1 and Table I. These methylbranched analogues can also be found in the cuticulary waxes of the Cistus plants [26].

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