Further Oxygenated Compounds in the Essential Oil of *Cistus ladanifer* L. (*Cistaceae*)

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Benzyl benzoate, cis-ocimenone and a new acetophenone derivative, 2-hydroxy-6-methyl acetophone, could be isolated by chromatographic methods from the essential oil of *Cistus ladanifer*. Structural elucidation by NMR and MS are described. In addition pinocarvone, campholene aldehyde and tagetone were identified by their mass spectra.

Introduction

Essential oils from some species of the mediterranean genus *Cistus* show a very complex composition; they consist of several hundred compounds [1–3]. The isolation and identification of phenylpropanoic acid esters from the essential oil of *Cistus ladanifer* has been reported in a previous paper [4].

In this communication the occurrence of six further oxygenated compounds, previously unknown in the essential oil of *Cistus ladanifer*, is reported, benzyl benzoate, cis-ocimenone, 2-hydroxy-6-methyl acetophenone, pinocarvone, campholene aldehyde and tagetone. Benzyl benzoate has already been found in essential oils and plant aromas [5–9]. The occurrence of cis-ocimenone in essential oils was rarely reported [10]. 2-Hydroxy-6-methyl acetophenone has thus far not been described. Pinocarvone, campholene aldehyde and tagetone have previously been found in essential oils [10–12], but have thus far not been reported for that of *Cistus ladanifer* [19].

Materials and Methods

The *Cistus* plants were grown from seeds and cultivated in the field of the Botanical Institute of the University of Köln. Extraction of essential oils was carried out by steam destillation. Fractionation of the oil according to functional groups was achieved by SiO_2 column chromatography [1, 2, 4]. Ester and carbonyl compounds were eluted with 2-chloropropane. This fraction was further separated into four fractions by preparative TLC (TLC-plate 40 × 20 cm, SiO_2 60 GF_{254} from Merck, Darmstadt, layer 0.5 mm), solvent system petrol ether/1,2-dichloroethane (2:1 v/v).

Fractions with R_f-values 0.27 contained benzyl benzoate, 0.22 *cis*-ocimenone and 0.11 2-hydroxy-6-methyl acetophenone. Benzyl benzoate and *cis*-ocimenone were further purified by repeated TLC on SiO_2-plates (TLC-plates 20 × 20 cm, SiO_2 60 G from Merck, Darmstadt, layer 0.25 mm), impregnated with 1 g $AgNO_3$ being dissolved in 10 ml acetonitrile, solvent systems 1,2-dichloroethane. The acetophenone derivative was purified by repeated TLC on SiO_2-plates (TLC-plates 20 × 20 cm, SiO_2 60 GF_{254} from Merck, Darmstadt, layer 0.25 mm), solvent system ether/*n*-pentane (95:5 v/v). Detection of these compounds on TLC-plate was achieved by UV (254 nm) or by spray reagent dichlorofluoresceine. Benzyl benzoate was hydrolysed with 0.5 N KOH for one hour under refluxing, the free benzoic acid was methylated with diazomethane.

GC: Hewlett-Packard, model 5830 A with 18850 A GC-Terminal; glascapillary-columns:

stat. phase	temperature
10 m Sp 2100	50 °C–170 °C, 30′ isotherm
25 m FFAP	50 °C–220 °C, 50′ isotherm
25 m OV 101	50 °C–250 °C, 10′ isotherm
	rates 4 °C/min

IR: Perkin-Elmer, model Infracord 137, film between KBr-pellets. NMR: 60 MHz: Hitachi Perkin-Elmer, model R-24 B. 90 MHz: Varian, model EM 390. GC-MS: Finnigan, model 3200, electron energy 70 eV (EI).

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Results and Discussion

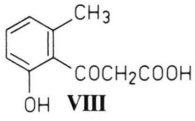
Three thus far not identified compounds in the essential oil of *Cistus ladanifer* could be isolated by chromatographic methods. The IR-spectrum of benzyl benzoate indicates the monosubstituted aryl ester (1750 cm^{-1}, strong: ester; 1500 cm^{-1}, medium: aromatic ring; 750 and 700 cm^{-1}, strong: monosubstituted benzene ring). The identification of the alcohol (**I**) and the acid (as methyl ester **II**) after saponification was achieved by mass spectrometry. The fragmentation patterns agree well with data given in the literature [13–17] (Table I).

The IR-spectrum of *cis*-ocimenone (**III**) indicates the unsaturated carbonyl (1680 cm^{-1}, strong). The identification as ocimenone could be achieved by mass spectrometry. The fragmentation pattern agrees well with data published before [10] (Table I). Since *cis/trans*-ocimenone cannot be distinguished mass spectroscopically the identification of the *cis*-isomer was achieved by NMR-spectroscopy. These data agree also with the literature [10]. Pure *cis*-ocimenone was found to be very instable. Partial rearrangement to *trans*-ocimenone was shown to occur by GC and NMR.

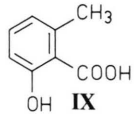
2-Hydroxy-6-methyl acetophenone (**IV**) was identified as aromatic ketol by its IR-spectrum (3300 cm^{-1}, strong: hydroxyl group; 1680 cm^{-1}, strong: unsaturated carbonyl; 1500 cm^{-1}, medium: aromatic ring). The structure of (**IV**) follows from the NMR-spectrum (Table I). The signal of the hydroxyl proton was found at 12 ppm. The two sharp singlets at 2.6 and 2.7 ppm stem from the methyl groups a and b. The signals of the three aromatic protons c, d, e, are found at 6.7 ppm, 6.9 ppm and 7.3 ppm and are split to two doublets and one double doublet.

Interpretation of these signals could be verified by increment calculation. This acetophone derivative has thus far not been described in essential oils.

Biogenetically, **IV** is a member of the polyketide group. Cyclisation of a C_{10}-unit would lead to **VIII** which being a β-keto acid that could readily decarboxylate. **VIII** is the pentaketide analogue of 6-methyl salicyclic acid (**IX**) for which it has been shown by labelling studies that the aromatic methyl group is the CH_3-terminus of $CH_3(COCH_2)_nCOOH$ [18].



VIII



IX

The presence of three additional carbonyl compounds is indicated by their mass spectra: pinocarvone (**V**), campholene aldehyde (**VI**) and tagetone (**VII**). Comparison spectra were kindly supplied by Dr. B. Willhalm (Firmenich, S. A., Geneva) [19]. In Fig. 1 is shown the gas liquid chromatogramm of the esters, carbonyl compounds and ethers obtained from *Cistus ladanifer*. The GLC was recorded on a glass capillary column Sp 2100 and demonstrates the complexity of the essential oil of *Cistus ladanifer*.

Known compounds (*cf.* refs. [1, 2, 4] and this paper) have been marked as such.

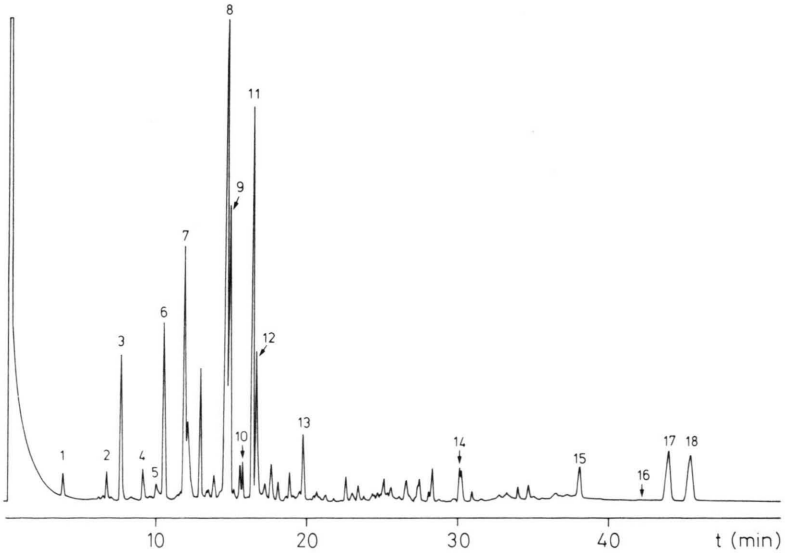
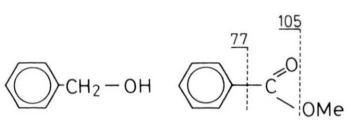
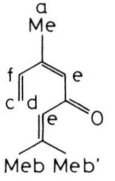
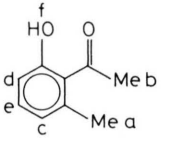


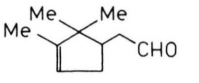
Fig. 1. GLC of 2-chloropropane fraction from *Cistus ladanifer*. GLC analysis was run on Sp 2100 glas capillary column. 1. Benzaldehyde; 2. 2,2,6-trimethyl cyclohexanone and 1,8-cineole; 3. acetophenone; 4. (±) fenchone; 5. thujone; 6. campholene aldehyde; 7. menthone, pinocarvone and tagetone; 8. *cis*-ocimenone; 9. neral and *trans*-ocimenone; 10. geranial; 11. bornyl acetate; 12. 2-hydroxy-6-methyl acetophenone; 13. geranyl acetate; 14. benzyl benzoate; 15. phenylethyl phenylpropanoate; 16. phenylpropyl phenylpropanoate; 17. geranyl phenylpropanoate; 18. dehydrogeranyl phenylpropanoate.

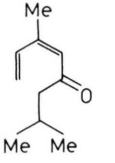
Table I. Structure and spectral data from isolated oxygenated compounds.











I		**II**		**III**					**IV**					**V**		**VI**		**VII**	
MS (EI)		MS (EI)		NMR [b]				MS (EI)		NMR			MS (EI)		MS (EI)		MS (EI)		MS (EI)	
m/e	int % [a]	*m/e*	int %	H	δ	*J*	*I*	*m/e*	int %	H	δ	*J*	*I*	*m/e*	int %	*m/e*	int %	*m/e*	int %	*m/e*	int %
108 M^+	65	136 M^+	28	a s	1.9	–	3	150 M^+	20	a s	2.6	–	3	150 M^+	50	150 M^+	5	152 M^+	2	152 M^+	2
107	50	105	100	b s	2.1	–	3	135	90	b s	2.7	–	3	135	100	135	25	137	5	137	5
91	15	77	85	b′ s	2.2	–	3	107	45	c d	6.7	8	1	107	15	122	15	119	22	110	5
90	8	51	45	c d	5.2	10	1	105	15	d d	6.9	8	1	79	40	108	80	109	22	109	10
89	9			d d	5.5	17	1	95	30	e dd	7.3	8	1	77	55	107	50	108	100	96	10
79	100			e s	5.9	–	2	91	55	f s	12.0	–	1	51	25	91	15	95	35	95	100
77	62			f dd	6.3	10	1	83	42							81	100	93	85	67	25
51	32					17		79	40							79	40	91	25	65	5
								77	20							77	20	81	15		
								67	58							69	15	79	12		
								55	100							53	60	77	15		
																		67	20		

[a] Relative intensity in %.
[b] NMR-spectrum III was recorded at 60 MHz, spectrum IV at 90 MHz ($CDCl_3$ or CCl_4, TMS) δ-values in ppm, *J*-values in Hz, *I* number of protons.

Acknowledgements

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K. Nabeta, A. Ichihara, and S. Sakamura, ibid. p. 409,
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[19] B. Willhalm (Firmenich, S. A., Geneva) informed us (letter from March 6, 1980) that these three compounds have also been isolated from *Cistus* species in his company (S. Escher, unpublished).