

Epicuticular Leaf Wax of *Cistus albanicus*, Cistaceae

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The epicuticular wax of *Cistus albanicus* was investigated. Homologous series of alkanes, wax esters, alcohols and long chain fatty acids were identified. The alcohol fraction consisted of primary and most probably of secondary alcohols.

Introduction

Cistus albanicus E. F. Warburg ex Heywood is a low perennial shrub of endemic occurrence in Albania and in the north-west of Greece. It has been described as a new *Cistus* species only recently [1, 2]. The leaf surface of the aerial parts of the genus *Cistus* is covered with a waxy material and in addition with a resin secreted by glandular trichomes. The epicuticular waxes are a mixture of lipophilic hydrocarbons, wax esters, alcohols and fatty acids and are most important as a water and gas diffusion barrier.

To continue our investigations of *Cistus* waxes [3–6] we analysed *C. albanicus* in regard to its wax constituents.

Material and Methods

Plant material was cultivated from seeds at the Botanical Institute of Cologne under natural garden conditions. 122 g of fresh plant material were washed with CHCl_3 and yielded 2.8 g of epicuticular material, with 6.9% of dry weight. This was dissolved in hot MeOH and subsequently cooled down to -20°C to precipitate and separate the wax from the terpenoids (2.2 g). The MeOH insoluble wax (0.38 g; 0.94% of the dry wt.) was fractionated on silica gel columns (Merck 60) as reported [7, 8]. Analysis of the fractions by TLC on silica gel plates and the chemical reactions such as reduction, ethanolsis of the wax esters, acetylation of the alcohols and methanolysis of the free fatty acids as well as identification by GC followed the usual procedures [7, 8].

Analysis of the alcohols was achieved by additional GC/MS data.

Results and Discussion

The composition of *C. albanicus* leaf wax is summarized in Table I. Main components are the wax esters (0.33% of dry wt.) with chain lengths ranging from C_{36} to C_{56} . Hydrocarbons (0.17% of dry wt.) were found to range from C_{19} to C_{39} with nonacosane as the main component. These results are in accordance with results obtained from other *Cistus* species [6]. No unsaturated hydrocarbons could be detected.

In the MeOH fraction fatty acids (0.08% of dry wt.) were found and identified as their methyl esters, which ranged from C_{14} to C_{32} . Additionally this fraction yielded primary alcohols (0.20% of dry wt.) even numbered and with chain lengths from C_{20} to C_{30} .

Together with the primary alcohols, the GC data showed peaks of homologous series of yet unknown components. They were always found to occur shortly after the alcohol peaks. They could be acetylated, but not hydrogenated and showed chromatographic behaviour just as the detected primary alcohols. Yet TLC on silica gel plates with solvents $\text{CH}_2\text{Cl}_2:\text{EtOAc}$ (24:1) showed two spots with R_f 0.34 for primary alcohols and R_f 0.39 for secondary alcohols. The small difference between these spots indicated secondary alcohols (R_f 0.39) with OH-position close to the beginning of the C-chain, possibly in position 2.

GC/MS data showed two characteristic fragments: M-18, which is typical for alcohols in general (loss of water), and a fragment with m/z 45 (basic fragment) which is produced by $\text{CH}_3 - \text{CH} = \dot{\text{O}} - \text{H}$. This fragment typical for secondary alcohols with an OH group in position $\Delta 2$ and in accordance with other data, strongly hints to the presence of these compounds in the leaf wax of *C. albanicus*. The secondary alcohols were uneven in chain length ranging from C_{23} to C_{31} with pentacosan-2-ol dominating. The results are the first indication for the presence of secondary alcohols in the leaf wax of the genus *Cistus*.

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Table I: Quantitative composition of epicuticular wax components of *C. albanicus* (peak area %).

No. of C-atoms	Alkanes	Alcohols Free prim. sec.	Esterified	Fatty acids Free	Esterified	No. of C-atoms	Wax esters
14				2.6		36	0.3
16:0				6.4		37	+
16:1				0.3		38	6.1
18:0			1.2	1.8	1.7	39	0.5
18:1				2.0		40	5.7
20:0	4	1.5	2.7	14.5	29.0	41	0.6
20:1				0.5		42	19.3
21	+			0.2		43	2.4
22	+	10.8	57.9	12.1	28.6	44	22.4
23	7.1	2.0		0.5		45	2.2
24	0.2	18.2	24.7	15.7	21.2	46	18.6
25	2.4	37.8		4.3		47	2.1
26	0.2	7.2	8.3	10.5	8.3	48	11.3
27	14.0	15.5		3.0		49	1.1
28	2.3	2.5	3.5	9.9	1.2	50	4.6
29	55.1	4.5		1.1		51	0.4
30	1.5	+	1.7	13.1	0.8	52	2.2
31	14.1	+		+		53	+
32	0.3			1.4		54	0.3
33	1.8					55	+
34	4.0					56	+
35	0.7						
36	4.0						
37	0.3						
38/39	+/+						
Yield in mg	70	80		32		135	
in % dry wt.	0.17	0.20		0.08		0.33	

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