Esters of Benzyl Alcohol and 2-Phenyl-ethanol-1 in Epicuticular Waxes from *Jojoba* Leaves

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Z. Naturforsch. **41c,** 673–676 (1986); received April 4/April 30, 1986

Jojoba, Simmondsia chinensis, Benzyl Acyl Esters, 2-Phenyl-ethyl-1-acyl Esters, Fatty Acids

Esters of very long chain fatty acids with benzyl alcohol and the homologue 2-phenyl-ethanol-1 were found in the epicuticular waxes from *Jojoba (Simmondsia chinensis)* leaves. The isolation of these esters by chromatographic methods, the structural elucidation by GC-MS and their chemical reactions and synthesis are described.

Introduction

Jojoba (*Simmondsia chinensis* (Link) Schneider) is a desert scrub, native to the sonoran Deserts in the United States and Mexico. This plant is cultivated in plantations around the world in areas of semiarid climate to produce a seed oil of high qualities which consists of unsaturated wax esters unique in the plant kingdom [1].

The epicuticular waxes of *Jojoba* leaves consist of hydrocarbons, wax esters, aldehydes, alcohols, fatty acids and also esters of very long chain fatty acids with yet unknown short chain alcohols as described earlier [2–5]. In order to elucidate the structure of these esters the *Jojoba* leaf wax was reanalyzed. The isolation and identification of these esters as well as further compounds is reported in this communication.

Material and Methods

Plant material

Jojoba leaves were harvested a) at their natural habitats in Arizona, USA, from five different locations and b) from a plantation of the Negev Jojoba Company in Beer-Sheva, Israel.

Isolation

The air dried whole leaves (44.8 g) were extracted with chloroform. Fractionation of wax components (149 mg) was achieved by silica gel (Merck 60) column chromatography with the solvents pentane, 2-chloropropane and methanol as described earlier [2, 4, 5] but only the substances soluble in hot hexane (50 ml) were chromatographed. The insoluble part was added to the methanol fraction.

Methanolyses

The substance was refluxed with 2 N HCl/MeOH for 2 h. After evaporation of the solvent the residue was dissolved in CH_2Cl_2 and reduced to dryness once more to completely remove the HCl. Finally the product was dissolved in hexane and analyzed.

Reduction

A solution of the compounds in benzene was stirred with $NaBH_4$ deposited on alumina [6] for 2 h.

TLC

Silica gel precoated plates (Merck 60), and benzene as eluent were used.

GC

Hewlett Packard model 5710 A with FID and integrator 3380 S. 20 m glas capillary column OV 101; Temp. 180 °C to 320 °C, rate 4 °C/min.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0700–0673 $ 01.30/0

GC-MS

Finnigan-MAT 4510; 70 eV; EI.

MS data

a) Esters I

Benzyl tetracosanoate: *m/z* (rel. int.): 367 (M^+ – 91, 1.5); 91 (100); 108 (88); 43 (45); 57 (40); 125 (2).

Benzyl hexacosanoate: 486 (M^+, 0.2); 395 (M^+ – 91, 0.5); 91 (100); 108 (85); 43 (45); 57 (40); 125 (3).

Benzyl octacosanoate: 514 (M^+, 0.1); 423 (M^+ – 91, 0.4); 91 (100); 108 (85); 43 (48); 57 (45); 125 (3).

Benzyl triacontanoate: similar data.

b) Esters II

2-Phenyl-ethyl-1-docosanoate, 2-phenyl-ethyl-1-tetracosanoate, 2-phenyl-ethyl-1-hexacosanoate, 2-phenyl-ethyl-1-octacosanoate, 2-phenyl-ethyl-1-triacontanoate.

All esters show the same mass fragments, no $(M)^+$ but a very strong base fragment; *m/z* (rel. int.): 104 (100); 43 (12); 57 (8); 71 (5).

Synthesis of benzyl acyl esters (esters I) and 2-phenyl-ethyl-1-acyl esters (esters II).

A mixture of hexacosanoic acid and octacosanoic acid (100 mg each) was added to an excess of a) benzyl alcohol or b) 2-phenyl-ethanol-1 and a few drops of H_2SO_4. The reaction was carried out by heating the mixture to 80 °C for 1 h. The reaction products were extracted with ether/petrol ether, washed for neutrality, dried over $MgSO_4$, filtered and evaporated. The esterification was checked by TLC. The synthetic esters I and II were purified on a silica gel column as described for the natural products. Esters I were additionally purified by TLC.

Esters I: benzyl hexacosanoate, benzyl octacosanoate.

Esters II: 2-phenyl-ethyl-1-hexacosanoate, 2-phenyl-ethyl-1-octacosanoate.

Results and Discussion

The epicuticular waxes, extracted with chloroform from *Jojoba* leaves, were fractionated on a silica gel column with the solvents pentane, 2-chloropropane and methanol as described [2, 4, 5]. The separation procedure was modified by applying only that part of the wax to the column which is soluble in hot hexane. The insoluble residue consists mainly of long chain fatty acids and was added to the methanol fraction. In contrast to an earlier publication [2], the yield of very long chain fatty acids in *Jojoba* was now increased up to 41%. Additionally, losses during chromatography were reduced. The proportion of very long chain fatty acids in the wax was increased and octacosanoic acid (52.8% of the total acids) was found to be the main component. The fatty acid pattern differs considerably from that published in [2]. The pattern of primary alcohols did not change with the modified fractionation.

With 2-chloropropane as solvent, a very complex mixture (22 mg) of different compounds was eluted from the silica gel column. The occurrence and the composition of wax esters and aldehydes in this fraction was confirmed, but the presence of acetates could not be confirmed. Instead, a homologous series of five components was isolated by TLC (R_f 0.56) which was always associated with the above mentioned esters.

Several features proved these five compounds to be homologous ketones with chain lengths of C_{25}, C_{27}, C_{29}, C_{31}, and C_{33}. Thus they showed the typical GC- and TLC-behaviour of long chain ketones and they were reducible with $NaBH_4$ to form the corresponding secondary alcohols (R_f 0.26) which in turn could be acetylated.

The fatty acid components of the esters of long chain fatty acids and short chain alcohols previously reported to occur in *Jojoba* leaf wax [2–5] had been analyzed to range from C_{22} to C_{30}, whereas the alcohol components had not yet been identified.

With the aid of preparative TLC on silica gel plates and benzene as eluent we were able to isolate two bands which were found to contain these esters. The band with R_f 0.46 contained one homologous series of esters (esters II) and was always associated with aldehydes, the band with R_f 0.52 contained a second series (esters I) and was associated with ketones. In order to separate aldehydes and ketones from the esters they were reduced with $NaBH_4$ and the reduction products were fractionated on a silica gel column. With 2-chloropropane as eluent, pure fractions of esters I (R_f 0.52) and esters II (R_f 0.46) were eluted (see Fig. 1). Esters I and II were saponified separately. The resulting fatty acids, analyzed as their methyl esters, are listed in Table II. Yield and composition of esters I and II as well as of the other compounds were calculated from GC chromato-

Table I. Yield and composition (peak area %) of the 2-chloropropane fraction from *Jojoba* leaf wax. This fraction consists of aldehydes, ketones, benzyl acyl esters (I), 2-phenyl-ethyl-1-acyl esters (II) and wax esters.

C No.	Aldehydes	Ketones	Esters I + II	C No.	Wax Esters
22	+			36	+
23	0.2			37	+
24	2.2			38	6.5
25	2.3	10.5		39	0.6
26	7.3			40	23.5
27	5.3	30.3		41	1.4
28	34.0			42	24.2
29	3.7	27.7		43	1.4
30	26.6		0.7	44	20.3
31	1.4	25.4	0.7	45	1.2
32	16.3		22.2	46	14.5
33	0.7	6.1	4.1	47	0.3
34	+		29.4	48	5.6
35			10.8	49	+
36			29.9	50	0.6
37			0.7	51	+
38			1.2	52	+
				53	+
				54	+
R_f value*	0.44	0.56	0.46 II 0.52 I		0.64
% fraction	48.3	9.9	21.8		19.9

* TLC: Silica gel, benzene.

Table II. Fatty acids and alcohols of benzyl acyl esters (I) and 2-phenyl-ethyl-1-acyl esters (II).

GC	TLC	Esters I + II	Fatty acids	Alcohols	
				Esters I	Esters II
RT	R_f	C No.	C No.	C No.	C No.
15.4	0.46	30	22		8
16.7	0.52	31	24	7	
18.2	0.46	32	24		8
19.5	0.52	33	26	7	
21.0	0.46	34	26		8
22.5	0.52	35	28	7	
23.6	0.46	36	28		8
25.0	0.52	37	30	7	
26.4	0.46	38	30		8

grams of the total 2-chloropropane fractions of extracts from *Jojoba* leaves harvested in Beer-Sheva, Israel (Table I). The preparation of single substances by TLC is often accompanied by quantitative disproportions of the analyzed compounds.

GC-MS analysis of the two ester series revealed benzyl alcohol and 2-phenyl-ethanol-1 as alcohol

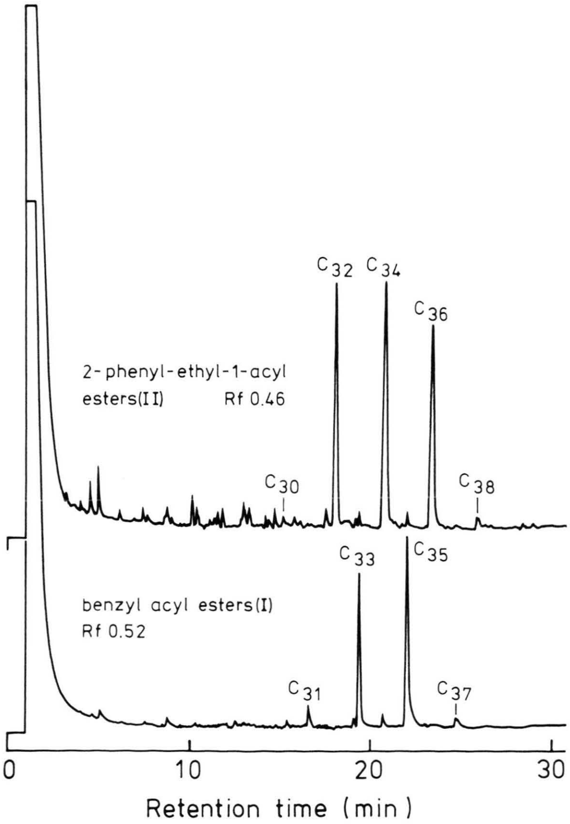

Fig. 1. GC of esters I and II, isolated by preparative TLC on silica gel.

components as seen by their dominant fragment ions *m/z* 91 and *m/z* 104, respectively [7]. The coordination of these alcohols to their corresponding fatty acids is compiled in Table II. The structure of the esters was verified by synthesis. Thus synthetic benzyl acyl esters (I) with acid chain lengths of C_{26} and C_{28}, and esters II from 2-phenyl-ethanol-1 and the same fatty acids showed the same chromatographical behaviour and MS data as the isolates from *Jojoba* leaves.

The structures of esters I and II are given in Fig. 2. These esters were isolated from *Jojoba* leaves for the first time and have not yet been described for any other epicuticular wax. They seem to be general constituents of *Jojoba* leaves, because they were found in plants of five different habitats in Arizona, USA, as well as in a plantation in Beer-Sheva, Israel.

Other esters of both alcohols were shown, however, to occur in the essential oils from *Cistus* leaves, such as benzyl benzoate [8] and 2-phenyl-ethyl-1-phenylpropanoate [9].

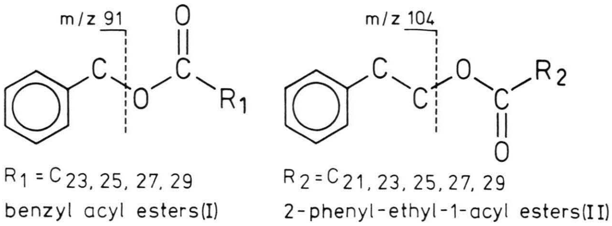

Fig. 2. Structures of esters I and II.

Acknowledgements

The authors thank Prof. J. Wisniak and the Negev Jojoba Company in Beer-Sheva, Israel, for sending *Jojoba* plant material, Dr. P. Proksch for harvesting *Jojoba* leaves at their natural habitats in Arizona, USA, and Mrs. E. Müller for very skilful assistance.

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