Composition of Epicuticular Waxes from Fruits of *Jojoba* (*Simmondsia chinensis* [Link] Schneider)

Paul-Gerhard Gülz

Botanisches Institut der Universität zu Köln, Gyrhofstr. 15, D-5000 Köln 41, Bundesrepublik Deutschland

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Epicuticular waxes were extracted with hexane from dry *Jojoba* pericarp and seed. These cuticular waxes consisted of hydrocarbons, wax esters, free acids, free alcohols and sterols; additionally aldehydes were found in the wax obtained from seed coats. The hydrocarbon fraction contained a homologous series of *n*-alkanes and branched alkanes but no alkenes. The composition of the wax esters of the cuticular wax was similar to that of the cotyledons. The esters are composed of monounsaturated long chain acids and alcohols.

Introduction

Jojoba (*Simmondsia chinensis* [Link] Schneider) is a shrub native to the arid regions of the Sonoran desert in southwest North America. The last year showed a growing interest in *Jojoba* plants especially its seed oil [1, 2]. *Jojoba* seeds accumulate wax esters which account for more than 50% of their dry weight and which are localized in the cotyledons. These wax esters serve as storage lipids in contrast to other plants where triglycerides are used for storage material. Homologous series of wax esters with predominantly even numbered, straight and long carbon chains (C_{32} to C_{48}) were analysed (3–5). The esters result from a combination of monoenic acids and alcohols with chain lengths ranging from C_{16} to C_{26}. The alcohols and fatty acids are mostly unsaturated with the double bond in the ω-9 position (6). As a result, these unsaturated wax ester mixtures are of liquid consistency. These wax esters have the function of storage lipids just as the triglycerides in other plants and they are metabolized during the germination of *Jojoba* seeds for gluconeogenesis and energy metabolism (7–9). Primarily, wax esters are known as components of cuticular waxes of all land plants (10–12). In the following study the epicuticular waxes of *Jojoba* fruits were isolated and analysed.

Materials and Methods

Jojoba fruits were collected in August 1981 from their natural habitat near Tonto National Monument, Arizona, USA. The air dried fruits were separated into pericarp and seed.

40.4 g pericarp (90.7% dry wt) and 124.25 g seeds (94.4% dry wt) were extracted three times with hexane for a total of 9 min. The resulting wax was fractionated on a silica gel 60 (Merck) column. The hydrocarbons were eluted with pentane; esters and aldehydes with 2-chloropropane; and free alcohols and free acids with methanol. The yield and the composition of the waxes are summarized in Table 1. Separation of hydrocarbons into *n*-alkanes and branched alkanes are obtained with Linde's molecular sieve type 5 A (13). The wax esters were converted into ethyl esters with 5% HCl in anhydrous ethanol. Free fatty acids were esterified with 5% HCl in anhydrous methanol. Alcohols were acetylated with pyridin and acetic anhydride. Aldehydes were reduced to primary alcohols with $NaBH_4$ in dioxane/water (4 : 1).

TLC: TLC plates were coated with silica gel 60 (Merck). Solvent system: 1) benzene for wax esters, aldehydes and esters; 2) dichloromethane/ethylacetate (96 : 32) for free fatty acids, alcohols and sterols. The spray reagent was bromothymolblue.

GLC: Hewlett-Packard model 5750 with FID and integrator 3380 S. 25 m glass capillary column DUHT-OV-101 (Macherey-Nagel & Co.) splitless, pressure 1.3 kg/cm^2 N_2.Temp.program: 1) 140 °C–240 °C; 4 °C/min for hydrocarbons, sterols and alcohols. 2) 180 °C–300 °C; 4 °C/min for wax esters, aldehydes and esters. Hewlett-Packard model 5830 with FID. 10 m glass capillary column FFAP, split rate 1 : 8 N_2. Temp.program: 140 °C–220 °C; 4 °C/min for fatty acid methyl esters and alcohols.

Reprint requests to Dr. P.-G. Gülz.

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Results and Discussion

Whole seeds were washed three times with hexane. Further extractions including hot hexane resulted in no further yield of waxes. Thus it may be concluded that only waxes from the seed coat are extracted by this method and not lipids from other parts of the seed. This is confirmed by the very low yield of waxes in amounts of 0.03% of dry wt of total seed. Waxes were also isolated from the seed coat as well as from the pericarp of *Jojoba* fruits. These waxes differed qualitatively and quantitatively from each other (Table I). In the wax from the seed coat very high concentrations of wax esters were found and additionally aldehydes. These aldehydes do not occur in the pericarp.

Hydrocarbons

For separation, waxes were placed on a silica gel column. Only hydrocarbons could be eluted with pentane. GLC of these fractions showed a very complex mixture of hydrocarbons with chain lengths ranging from C_{19} to C_{35}.

Normal alkanes were observed in a homologous series as well as branched alkanes. High purity (98%) of these branched alkanes could be achieved by using Linde's molecular sieve type 5 A. Hydrocarbons from the pericarp contain 30% branched alkanes; from the seed coats 45%. The peak areas per cent of these fractions are listed in Table II. The retention times in the GLC of the branched alkanes indicate a predominance of 2-methyl-alkanes and small amounts of 3-methyl-alkanes (13). No alkenes could be observed in the hydrocarbon fractions. TLC with $AgNO_3$ impregnated plates showed no separation of these fractions.

Table I. Composition and yield of cuticular waxes from *Jojoba* fruits.

	Pericarp		Seed coat	
	[mg]	% wax	[mg]	% wax
hydrocarbons	5	20	1	3
wax esters	2	8	14	42
aldehydes	–	–	2	6
unidentified	–	–	2	6
alcohols	6	25	4	12
sterols	4	17	2	6
free acids	4	17	2	6
lost on column	3	13	6	18
wax	24 = 0.07% dry wt		33 = 0.03% dry wt	

Table II. Composition (peak area %) of hydrocarbon fractions from *Jojoba* fruits.

No. of C-atoms	Pericarp		Seed coat	
	n-alkanes	br. alkanes	*n*-alkanes	br. alkanes
19	+		+	
20	+		+	
21	+	+	+	+
22	+	+	+	+
23	0.3	+	0.5	+
24	0.3	+	0.6	+
25	1.5	0.4 i	2.3	1.2 i
26	0.8	0.4 a	1.2	0.7 a
27	10.8	1.5 i	9.1	3.5 i
28	2.0	1.3 a	2.3	2.2 a
29	21.2	3.9 i	14.4	12.2 i
30	2.2	4.7 a	2.8	6.0 a
31	27.9	9.7 i	19.4	13.5 i
32	1.0	6.8 a	1.3	3.9 a
33	2.0	1.3 i	1.6	1.3 i
34	+	+	+	+
35	+	+	+	+
	70.0	30.0	55.5	44.5

i = *iso* = 2-methyl.
a = *anteiso* = 3-methyl.

Note added in proof:

Mass-spectra of the branched alkanes show fragments typical for the presence of 2-methyl branchings in odd-numbered alkanes (fragment M-15 and M-43) and for the presence of 3-methyl branchings in even-numbered alkanes (fragment M-29). I thank Prof. Dr. H. Budzikiewicz (Köln) for recording and interpretation of the mass-spectra.

Wax esters from *Jojoba* seeds and also from seed coats consist predominantly of monenic acids and alcohols with ω-9-positioned double bonds. On the other hand, as mentioned above, in the hydrocarbon fractions of the same seed organs no alkenes were found, since only saturated *n*-alkanes and branched alkanes were determined. Therefore, it may be assumed, that the alkanes in *Jojoba* fruit pericarp and seed coat have not the same acid precursor as the wax esters of *Jojoba* seeds which utilize oleic acid. The site of biosynthesis of these substances as well as the reactions of chain elongation may be different for alkanes and wax esters, and furthermore they do not start a common acid pool. The enzymes for elongation will also be different. Those necessary for alkane biosynthesis may not be identical to those of the described pathway for *Jojoba* wax esters, their acids and alcohols (14–16).

Wax esters

Wax esters were eluted with 2-chloropropane from the silica gel column. In seed coats additional alde-

hydes were found in this fraction. The preparative separation of these two substances was achieved by TLC on silica gel with benzene as solvent. Wax esters (R_f 0.61) from seed coat and pericarp of *Jojoba* fruits consisted of unsaturated esters with chain lengths ranging from C_{32} to C_{48} and maximum at C_{40} and C_{42}. They were similar to the wax esters of *Jojoba* seeds. The saponification products were also similar to those described earlier (2). Among the resulting fatty acids of *Jojoba* seed coats eicosenoic acid was dominating with 66.3%. The major components in the alcohol fraction were eicosenol (43.2%) and docosenol (43.7%) (see Table III).

Aldehydes

Waxes from seed coats but not from the pericarp contain also aldehydes. They were identified by TLC (R_f 0.49), reduction with $NaBH_4$ to primary alcohols and by GLC in comparison with authentic samples. Aldehydes from seed coats were found to represent a homologous series with chain lenghts ranging from C_{24} to C_{30}. Octadecanal with 58.4% was dominating. The peak areas per cent are listened in Table II.

Free fatty acids

Alcohols and fatty acids were desorbed from a silica gel column with methanol. The fatty acids were esterified and the resulting fatty acid methylesters (R_f 0.44) were isolated by preparative TLC and then analysed by GLC. Free fatty acids consisted predominantly of saturated acids. Unsaturated fatty acids may account up to 20%. In this fraction no dominant acid was found in contrast to the saponification acids of wax esters (Table III). The composition of fatty acids from seed coats was similar to those of the pericarp.

Primary alcohols

Alcohols are identified in the methanol fraction as a homologous series of primary fatty alcohols. Several

Table III. Composition (peak area %) of epicuticular wax fractions from *Jojoba* seed coat.

No. of C-atoms	Free acids	Acids from wax esters	Alcohols from wax esters	Free alcohols	Aldehydes
12	0.5	0.3			
14	3.0	+			
15	1.2				
16	23.6	2.1		0.7	
16:1	3.6	1.1			
17	1.3			+	
16:3	0.6				
18	10.6	0.3	1.0	16.9	
18:1	9.8	12.0	0.8		
18:2	4.0	0.8			
19	0.6			0.3	
18:3	0.2	0.2			
20	4.9	0.3	0.7	41.0	
20:1	3.3	66.3	43.2		
21	+				
22	3.8	0.5	2.1	1.3	
22:1	1.1	11.7	43.7		
23	0.2			+	
24	13.2	0.4	1.1	1.9	3.5
24:1	+	1.0	6.9		
25	+			+	2.7
26	8.5			13.1	25.5
27				1.1	4.3
28	5.4			23.6	58.4
29				+	1.6
30				+	3.0
Cholesterol				3.0	
Campesterol				19.1	
Stigmasterol				5.0	
β-Sitosterol				72.9	

sterols were also found. Primary alcohols (R_f 0.49) could be separated from sterols (R_f 0.40) by preparative TLC with dichloromethane/ethylacetate (96 : 32) as solvent system. Alcohol acetates were analysed by GLC and are listed in Table III. The primary fatty alcohols consist predominantly of saturated alcohols, unsaturated alcohols are present only in traces. The alcohol patterns show two maxima with eicosanol (41.0%) and octacosanol (23.6%), and those from seed coats were similar to those from pericarps.

Sterols

The isolated sterols were identified by comparison with authentic samples (Supelco Inc.) by GLC. Cholesterol, campesterol, stigmasterol and β-sitosterol were present in proportions of 3 : 19 : 5 : 73 in the wax of seed coats as well as in the wax of the pericarp.

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