Jojoba Wax: Its Esters and Some of Its Minor Components

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A lack of reliability in the usual determinations of fatty acids and fatty alcohols of jojoba wax prompted us to propose an original method of hydrolysis and extraction, making it possible to better determine the composition of fatty acids and alcohols of the wax. High-performance liquid chromatography fractionation of the wax allowed isolation of four main classes of esters (which differed by their partition number). The detailed study of these ester classes emphasized the way acids and alcohols are connected, and fourteen distinct esters were thus identified. Some triacylglycerols, free fatty alcohols and other minor components of jojoba wax were found and quantitated. Seven sterols were identified, four for the first time.

KEY WORDS : Buxaceae, esters, fatty acids, fatty alcohols, HPLC, jojoba wax, *Simmondsia chinensis*, sterols, triacylglycerols.

Jojoba (*Simmondsia chinensis* Link, Buxaceae) is a perennial shrub that grows naturally in the deserts of Arizona and western Mexico. It is now cultivated in certain hot countries (Israel, some Mediterranean and African lands, South America).

It has long been known that the lipids extracted from jojoba seeds (40–65%) are mainly constituted of a wax that is a mixture of fatty acid and fatty alcohol esters. Jojoba wax has many uses related to its physical and/or chemical properties. It is a component of many cosmetic and pharmaceutical preparations, where it can replace spermaceti (1). Because it is not hydrolyzed by digestive lipases, it constitutes a noncaloric "fat" in dietetics. It can be used as a high-pressure lubricant or a neutral oil, owing to its low and almost constant viscosity over a large range of temperatures. Many other uses will be found when prices fall and production increases. Thus, the knowledge of its precise chemical composition is of utmost interest, particularly considering that synthetic substitutes (1) are coming on the market and must be distinguishable from the natural wax.

Qualitative and quantitative analysis of the fatty acids and alcohols of jojoba wax has been performed by many authors through different methods of hydrolysis or alcoholysis (2–14). A comparison of the published results shows that although similar values were found for acid composition, some discrepancies were observed in the composition of the alcoholic fraction. Such variations can be attributed either to differences in genetic variability or geographical origin of the seed or to inadequate methods of hydrolysis and/or extraction of the fatty alcohol fraction. Our first task was to determine the best hydrolysis procedure. Our second purpose was to determine precisely the esters present in the wax, that is to say, the way the plant associates acids and alcohols. Few authors (3,8) have studied this aspect. Our last objective was to confirm and complete the study of jojoba seed lipids by searching for minor constituents, such as acylglycerols, sterols and free fatty alcohols.

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MATERIALS AND METHODS

We used a pressure-extracted jojoba wax sample of commercial origin.

The high-performance liquid chromatography (HPLC) fractionation of jojoba wax and its hydrolysis products was performed on a Waters chromatograph (Waters Associates, Milford, MA) equipped with a 25-cm long, 4-mm i.d., reversed-phase RP 18-5 μm column (Lichrosorb; E. Merck, Darmstadt, Germany). Detection was done by refraction index (RI) variation (Waters). The mobile phase was propionitrile (E. Merck) with a flow rate of 0.8 mL/min. Four main peaks were observed with respective proportions of 7.1 (peak A), 31.4 (B), 51.7 (C) and 9.8% (D).

The acid-catalyzed ethanolysis of jojoba wax, with or without subsequent *in situ* acetylation of fatty alcohols, was performed according to several previously described methods (2,12). Ways of obtaining dry HCl/ethanol mixtures, acid concentrations and contact times were varied, but the HPLC analysis of the resulting mixture showed the presence of 8–35% of residual wax in all cases.

In the alkali-catalyzed hydrolysis procedure, a jojoba wax sample of about 50 mg was introduced into a 20-mL test tube with 0.1 mL benzene and 1 mL of a 30% solution of potassium hydroxide in 90% ethanol. The mixture, protected from atmospheric moisture by a calcium chloride trap, was refluxed for 30 min in an oil bath heated at 95°C. After addition of 10 mL anhydrous ethanol, the tube was allowed to cool to room temperature, and the solvent was then evaporated under reduced pressure. Then, 1-mL portions of anhydrous benzene were added and evaporated several times, until the residue was perfectly dry. This residue was left in contact overnight with 50 mL of methyl *tert*-butyl ether (MTBE) (E. Merck). The mixture was carefully filtered, giving a filtrate (fatty alcohols) and a solid residue (fatty acid potassium salts), which was then washed with MTBE.

The filtrate mixture was dried on sodium sulfate, filtered and evaporated to dryness. The residue, dissolved in a few drops of chloroform, was applied to 0.2-mm thick thin-layer chromatography (TLC) silica plates (E. Merck). Two elutions were performed with a hexane/diethyl ether (50:50, vol/vol) mixture. The fatty alcohol spot was scraped, and the resulting silica was extracted three times with hot chloroform. After filtration and evaporation, the alcoholic residue was acetylated overnight at room temperature by 5 mL of an acetic anhydride/pyridine mixture (50:50, vol/vol). After ordinary treatment, the hexane solution of fatty alcohol acetates was analyzed by gas chromatography (GC) on a DI 200 device (Delsi, Argenteuil, France) equipped with a flame-ionization detector, a Ross injector and a 25-cm long, 0.2-mm i.d. capillary column impregnated with Carbowax 20M (0.25-μm thick). The injector and detector temperature was 250°C, and oven temperature was 210°C. The vector gas was helium at a pressure of 1 bar.

The solid fraction was dissolved in water, acidified with 30% hydrochloric acid and extracted three times with MTBE. The organic layer was evaporated after drying on sodium sulfate. Then, 200 mg of residue was esterified by

methanol/boron trifluoride complex in accordance with IUPAC standards (15). The fatty acid methyl esters (FAME) were analyzed by GC under the same conditions as described previously.

The total reduction of jojoba wax by lithium aluminohydride was performed in the usual way (by Myher's method, Ref. 16), on 1 g of wax. The fatty alcohols thus obtained were acetylated and analyzed by GC under the same conditions as before.

The minor components could be obtained by chromatography of 5 g of wax on a 2 cm i.d. glass column filled with 150 g of 5% hydrated silica gel (E. Merck), dispersed in anhydrous hexane. Four fractions were obtained by successive elutions with hexane/diethyl ether (100:1, vol/vol) (fraction a, esters of fatty acids and alcohols), benzene (fraction b, triacylglycerols), diethyl ether (fraction c, free fatty alcohols; and fraction d, sterols). The purity of each fraction was checked by TLC on silica and by comparison with authentic samples (TLC solvents were hexane/ether mixtures: 100:1, vol/vol, for fraction a; 70:30, vol/vol for fraction b; and 30:70, vol/vol for fractions c and d). Two migrations were performed in each case.

After saponification of either fraction b or the initial wax, small quantities of glycerol could be detected and quantitated in the aqueous phase by enzymatic analysis (analytical kit for glycerol analysis provided by Boehringer-Mannheim S.A., Meylan, France).

GC analysis of sterols was performed on the same device as before, with a 25-m long, 0.32-mm i.d. capillary column filled with OV 1701 (0.25-μm thick). The oven temperature was 270°C, and that of the injector and detector was 350°C. The vector gas was helium at a pressure of 0.7 bar. HPLC analysis of sterols was performed with the same device as used before, with an RP18-5 μm column, an RI detector and anhydrous methanol as solvent at a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

All methods described in the literature by different authors to determine acids and alcohols associated in jojoba wax begin by an alkali or acid-catalyzed hydrolysis or ethanolysis of the esters, followed either by separate extractions of acidic and neutral fractions, or by a one-pot derivatization of the whole organic phase, acids being converted into FAME and alcohols into acetates. Identification and quantitation were done by GC on a polar column in all cases.

We reproduced several described methods of acid hydrolysis by using acetyl chloride/ethanol mixtures (12) or dry hydrogen chloride/ethanol solutions (2). We varied hydrochloric acid concentrations from 1.0–5.0 N and heating times from 10–90 min. The progress of the reaction was followed by HPLC on a reversed-phase (RP18) column. We observed a strong diminution of the four main peaks corresponding to initial esters, but we could not observe their total disappearance. In the best case (3), the remaining esters represented 8% of the initial wax. Moreover, a change in the relative areas of the four initial ester peaks was observed. This can be interpreted as the setting-up of an equilibrium between fatty esters, fatty acids ethyl esters (FAEE) and fatty alcohols. Moreover, FAEE and fatty alcohols recombine according to thermodynamic rules, introducing slight structural differences between the relative proportions of initial and remaining esters:

$$\underset{\text{(natural mixture)}}{\text{wax esters}} + \text{EtOH} \rightleftarrows \text{FAEE} + \text{fatty alcohols} \rightleftarrows \underset{\text{(thermodynamic mixture)}}{\text{esters}} \quad [1]$$

The usual treatment of the wax by aqueous alkali resulted in complete hydrolysis of the initial esters, as checked by HPLC. However, respective molar quantities of acids and alcohols determined by GC are not equal, with the quantity of extracted acids always being greater. In several successive experiments, we observed that GC analysis of the acid fraction always gave the same results, but experimental variations were observed in the total recovery of alcohols and in their relative proportions. This can be interpreted as bad recovery of fatty alcohols, related either to some solubility of alcohols in water or, more likely, to the formation of poorly extractable micellae of fatty alcohols in water.

This is the reason why we performed a longer, but improved method, of alkaline hydrolysis. The mixture was evaporated to dryness after saponification. Then, the alcohols could be completely extracted by MTBE, while acids were obtained by subsequent acidification and extraction. Equal quantities of acids and alcohols were thus obtained, with reproducible results, as summarized in Table 1.

To assess these results we have carried out a total reduction of the wax by lithium aluminohydride. The composition of the alcoholic mixture thus obtained is closely related to the values calculated from the previous analysis.

We then studied the true nature of the wax esters, i.e., the way the plant associated fatty acids and alcohols to form esters. HPLC analysis of the wax was performed under conditions that were similar to those used for triacylglycerol determination in glyceridic fats (17). Four main peaks (A, B, C and D), corresponding to four classes of esters, were thus obtained (Fig. 1). We supposed that, as for triacylglycerols, the retention time of each ester is related to its partition number (PN), i.e., the total number of carbon atoms minus twice the number of double bonds. Considering the possible esters of the wax, we assumed that the four classes corresponded to esters with PN values of 34, 36, 38 and 40, respectively.

The wax was quantitatively fractionated into four classes by HPLC. The fatty acids and alcohols of each

TABLE 1

Fatty Acid and Alcohol Composition of Jojoba Wax

Acids	%	Alcohols	%
C14:0	—	Tetradecanol	trace
C16:0 Palmitic	0.3	Hexadecanol	trace
C16:1 Palmitoleic	0.3	Hexadecenol	trace
C18:0 Stearic	0.2	Octadecanol	trace
C18:1 Oleic	9.3	Octadec-9-enol	0.6
C20:0 Arachidic	—	Eicosanol	0.4
C20:1 Eicos-11-enoic	76.7	Eicos-11-enol	45.4
C22:0 Docosanoic	trace	Docosanol	0.7
C22:1 Docos-13-enoic	12.1	Docos-13-enol	46.4
C24:0 Tetracosanoic	0.1	Tetracosanol	—
C24:1 Tetracos-15-enoic	1.0	Tetracos-15-enol	6.5

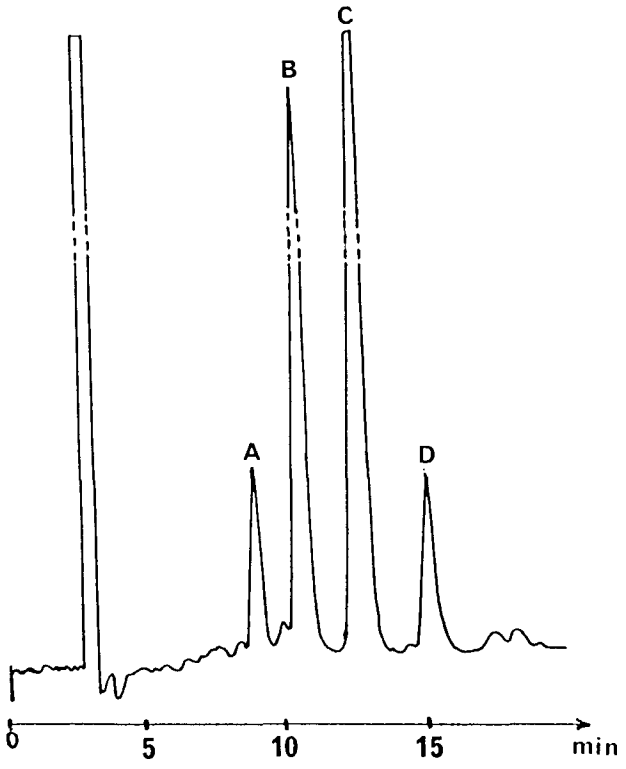

FIG. 1. High-performance liquid chromatography chromatogram of crude jojoba wax.

class of esters were identified and quantitated, according to the previous method. Then, in each class, acids and alcohols were associated in such a way that the resulting esters had the PN of the class. The results are given in Table 2.

The correlations between acids and alcohols were good, considering the experimental errors, which were within the limits of ±0.1%. Only once did we observe a discrepancy in the PN rule, namely, in the case of eicosanyl tetracosenoate (PN 42) found in the PN 40 class at a concentration of 0.9%.

From a biogenetic point of view, it is interesting that large differences occur between the observed results and those that can be calculated by a random association of acids and alcohols. For instance, we observed [acid/alcohol, % experimental (% random)]: [C20:1/C'20:1, 28.0% (31.8%)], [C20:1/C'22:1, 41.4% (32.0%)], [C22:1/C'20:1, 10.3% (5.7%)], [C22:1/C'22:1, 1.9% (5.7%)]. As frequently observed in natural products chemistry, the plant favors specific associations, which correspond to its needs and to its genetic program. From an analytical point of view, this observation constitutes a good discrimination between natural jojoba wax and its synthetic substitutes. In the latter case, associations between fatty acids and alcohols are governed by thermodynamic rules, and random results would be observed.

Spencer *et al.* and Miwa (3,8) are among the few authors who have also worked on jojoba wax ester identification by HPLC. The method they used consisted of an 8-hour acid ethanolysis of the sample, followed by saponification,

TABLE 2

The Esters of Jojoba Wax

Acid	Alcohol	Ester	%	Fraction	PN[a]
C16:0	C'20:1	Eicosenyl palmitate	0.3	A	34
C16:1	C'20:0	Eicosanyl palmitoleate	0.1	A	34
C16:1	C'22:1	Docosenyl palmitoleate	0.1	A	34
C18:0	C'18:1	Octadecenyl stearate	0.1	A	34
C18:1	C'20:1	Eicosenyl oleate	5.7	A	34
C18:1	C'22:1	Docosenyl oleate	3.4	B	36
C20:1	C'18:1	Octadecenyl eicosenoate	0.7	A	34
C20:1	C'20:1	Eicosenyl eicosenoate	28.0	B	36
C20:1	C'22:1	Docosenyl eicosenoate	41.4	C	38
C20:1	C'24:1	Tetracosenyl eicosenoate	6.8	D	40
C22:1	C'20:1	Eicosenyl docosenoate	10.3	C	38
C22:1	C'22:1	Docosenyl docosenoate	1.9	D	40
C24:1	C'20:0	Eicosanyl tetracosenoate	0.2	D	42
C24:1	C'20:1	Eicosenyl tetracosenoate	0.9	D	40

[a]PN, partition number.

and separated analyses of the acidic and neutral fractions. They considered that fractionation occurred according to molecular mass differences, but the practical results are the same because most acids and alcohols are monounsaturated.

Some minor components of jojoba wax could be identified either in the crude wax or in fractions obtained by column chromatography on silica (fractions a, b, c and d).

The presence of a small quantity of triacylglycerols was proved by TLC analysis of fraction b, which showed a spot with the same R_f as an authentic triacylglycerol sample. After saponification of the total wax, a quantitative analysis of glycerol gave a 0.14% concentration of triacylglycerol (assuming a medium molecular mass of 1,000). Identical results were obtained by a similar treatment of fraction b.

Fraction c contains only free fatty alcohols (0.8% of the wax) according to TLC and spectroscopic data (infrared, proton nuclear magnetic resonance). They were identified as acetates by GC. They are identical to the fatty alcohols found in esters, but their proportions (Table 3) are different. So, these free fatty alcohols, reported for the first time in jojoba wax, are not the result of random hydrolysis of esters.

The sterolic fraction (fraction d) which had been studied by Katoh *et al.* (18), constitutes 0.4% of the total wax. Using both GC and HPLC, we were able to distinguish at least nine different components, seven of which could be identified (Table 4). The three main sterols (sitosterol, campesterol and stigmasterol), which we identified by

TABLE 3

The Free Fatty Alcohols of Jojoba Wax

Alcohols		%	Total wax
C'18:1	Octadec-9-enol	5.3	0.6
C'20:0	Eicosanol	—	0.4
C'20:1	Eicos-11-enol	52.3	45.4
C'22:0	Docosanol	—	0.7
C'22:1	Docos-13-enol	38.4	46.4
C'24:1	Tetracos-15-enol	4.0	6.5

TABLE 4

The Sterols of Jojoba Wax

Sterol	Sterolic fraction (%)	Total wax (mg/kg)
Unidentified	0.2	8
Stigmasta-5,25-dien-3β-ol	0.6	24
Fucosterol	0.6	24
Isofucosterol	4.1	163
Unidentified	0.2	8
Cholesterol	0.8	32
Stigmasterol	6.7	266
Campesterol	16.9	672
Sitosterol	69.9	2780

mass and H nuclear magnetic resonance spectroscopies, have been previously mentioned for jojoba wax (18). Besides these compounds, we have proved the presence of cholesterol, isofucosterol, fucosterol and stigmasta-5, 25-dien-3β-ol by co-injections of the total sterolic fraction, both in GC and HPLC, with authentic samples otherwise studied in our laboratory. All compounds are common except the last one. Moreover, fucosterol is usually encountered in marine plants.

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