# OCCURRENCE OF LIPOPHILIC MICELLAE FORMED BY FATTY ALCOHOLS AND FATTY-ACID SODIUM SALTS IN JOJOBA-WAX AQUEOUS HYDROLYZATE

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## Introduction

Jojoba (*Simmondsia chinensis* [Link] Schneider, Buxaceae) is cultivated in certain warm countries for obtaining its seed oil comprising 45–60% of dry wt. The difference between jojoba oil and all other plant lipids is that this oil is not a triglyceride but a wax ester between very-long-chain fatty acids and higher fatty alcohols without the intervention of glycerol. This wax has numerous applications related to its physical and/or chemical properties. As a substitute for a hard-to-get sperm-whale wax, it is widely used as a component of skin-care, cosmetic, and pharmaceutical products, as well as an unmetabolized noncaloric "fat", and finds many industrial applications in preparing surfactants, high-pressure lubricants, protective coatings etc. (Wisniak, 1977). Therefore, several studies were devoted to the composition of jojoba wax (Hamilton and Raie, 1975; Spencer et al., 1977; Wisniak, 1977), but results of some of them have been rather contradictory (see below).

## Results and Discussion

In our work, while investigating the biosynthesis of this wax, it became essential to prepare tens-of-mg quantities of its fatty-acid and fatty-alcohol components. To this end, 170 mg of wax (Jojoba Bean Oil, Sigma-Aldrich Co., Cat. No. J-1375) was first purified by placing it on the 10 × 0.8 cm Woelm Silica Gel (Woelm, CCM, France) column and eluting with 8 ml of hexane. As a result, 165 mg of 99.6% pure wax was obtained.

It could be assumed that the most immediate approach to obtaining acids and alcohols from jojoba wax would consist of its direct saponification in an alkaline medium. For this purpose, we followed the instructions given in the standard textbook by Kates (1972), who recommended boiling of 30 mg of wax in a 0.3 N NaOH in MeOH-water (9:1) mixture for 1–2 h. However, in our experiments, refluxing of the jojoba wax in a 4% NaOH for up to 6 h failed to bring about complete hydrolysis of the ester bonds. Such results were consistent with those described earlier, when an exhaustive saponification of this wax was not achieved by using a 30% KOH solution or a 7-day-long hydrolysis with 1 M EtONa in EtOH (Miwa, 1971). It seems that the difficulties in performing alkaline hydrolysis of *S. sinensis* wax were caused by the fact that it almost totally consists of very-long-chain ($C_{38}$–$C_{46}$) aliphatic esters (Spencer et al., 1977).

Wax splitting can also be performed via its acid-catalyzed alcoholysis (Miwa, 1971) and some authors used to this end mild ethanolysis in the presence of HCl (Miwa, 1984). Subsequently, however, it was found that the products of this reaction,

regardless of its duration and HCl concentration, always contained considerable amounts (8% or more) of wax. In its ester composition, this wax notably differed from the initial one, and therefore it was concluded that it was synthesized in the reaction mixture itself, due to the setting-up of an equilibrium between wax esters, fatty acid ethyl esters, and fatty alcohols according to thermodynamic rules, which brought about wax resynthesis (Graille et al., 1986).

We suggested that such resynthesis could be abolished by replacing EtOH with a more polar alcohol, MeOH: their dielectric constants directly proportional to the polarity are equal to 24.3 and 32.6, respectively (Reichardt, 1973). Indeed, refluxing of the mixture of purified wax (150 mg), dry MeOH (10 ml), and AcCl (0.5 ml) for 120 min brought about, as shown by TLC, a complete conversion of the wax into fatty alcohols and fatty acid methyl esters.

Earlier, the saponification of very-long-chain fatty acid ethyl esters derived from jojoba wax required an overnight boiling with 1 N aqueous-alcoholic KOH solution (Miwa, 1971; Hamilton and Raie, 1975). Meanwhile, in our work, exhaustive saponification of their methyl esters with NaOH in MeOH : water (99 : 1) was achieved in only 60 min.

In order to separate wax unsaponifiables, including fatty alcohols, from the fatty-acid Na salts, Kates (1972) recommended the extraction of the alkaline hydrolyzate (see above) with 4 x 5 ml of petroleum ether, bp 30–60°C. However, as regards jojoba wax, our results showed that treatment of its hydrolyzate with either hexane or $Et_2O$ brought about a transfer in the organic phase not only of fatty alcohols, but also the bulk of fatty acids as their Na salts. As a result, this phase formed a stable opaque emulsion, which did not break for a long time.

Thus, a commonly used technique of separating unsaponifiables by the extraction of lipid-saponification products with nonpolar solvents proved inadequate with respect to jojoba wax. For establishing the reason of this phenomenon, it must be recognized that this wax differs from other plant lipids in the composition of its hydrolyzate, which consists of equal amounts of unsaponifiable (fatty alcohol) and fatty acid fractions; both of them include solely very-long-chain monounsaturated aliphatic moieties. Moreover, it has long been known that soap micellae have the power of holding oil-soluble materials in what is an equivalent to hydrocarbon solution between their long hydrocarbon chains (Markley, 1961). Therefore, we conclude that, in the aqueous-alkaline hydrolyzate of jojoba wax, fatty alcohols and fatty-acid Na salts formed stable lipophilic micellae highly soluble in hexane and $Et_2O$.

In order to break these micellae, we employed the technique of solid-phase extraction rather than the liquid-phase one. The products of alkaline hydrolysis of the jojoba wax methanolyzate were exhaustively dried *in vacuo* with heating, and the fatty alcohols were repeatedly washed out from the solid phase with dry $Et_2O$. Subsequently, this phase was acidified, and the fatty acids were recovered with hexane.

The purity of both preparation was assessed on the basis of the fact that a minimal weight of lipids, which could be visualized on a TLC plate with phosphomolybdic acid, was equal to 0.1 μg. This test showed that the preparations of fatty acids and fatty alcohols were 99.4 and 99.2% pure, respectively. The yield of their sum estimated gravimetrically amounted to 98.4% of the theoretical one.

Table 1. Fatty acid and fatty alcohol composition of jojoba wax

| Acids | % | Alcohols | % |
|---|---|---|---|
| Palmitic | 1.2 | Hexadecanol | 0.1 |
| Palmitoleic | 0.3 | Hexadec-7 enol | – |
| Stearic | 0.1 | Octadecanol | 0.2 |
| Oleic | 11.4 | Octadec-9-enol | 1.1 |
| Arachidic | 0.1 | Eicosanol | trace |
| Eicos-11-enoic | 71.5 | Eicos-11-enol | 43.8 |
| Docosanoic | 0.2 | Docosanol | 1.0 |
| Docos-13-enoic | 13.8 | Docos-13-enol | 44.9 |
| Tetracos-15-enoic | 1.4 | Tetracos-15-enol | 8.9 |

The composition of fatty acids as their methyl esters. and fatty alcohols determined by capillary GLC as described earlier (Pchelkin et al., 2001) is shown in Table 1. The results obtained were close to those found elsewhere (Miwa, 1971, 1984; Spencer et al.., 1977; Wisniak, 1977). Moreover, they were consistent with the evidence that all monounsaturated moieties of jojoba wax are characterized by ω9 structure (Spencer et al., 1977; Wisniak, 1977) and did not support the claims of Hamilton and Raie (1975), according to which the fatty alcohols of this wax contained double bonds at the ω4-, ω5-, ω6-, ω7-, ω8-, and ω9-positions.

Finally, our preliminary data suggest that fatty alcohols can be quantitatively separated from fatty acid methyl esters by single-solvent dry-column chromatography.

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