STRUCTURE OF THE ALCOHOLS DERIVED FROM WAX ESTERS IN JOJOBA OIL

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The monounsaturated C_{18}-C_{24} alcohols obtained by saponification of the wax esters of jojoba oil have been separated and the double bond positional isomers determined by a modified von Rudloff oxidation technique. The major homologue of each chain length has the double bond at the \( \omega \)9 position suggesting a close biogenetic relationship between these major components. The relationship is much less apparent in the minor components.

I. Introduction

The seeds of *Simmondsia Californica* Nutt, the evergreen desert shrub indigenous to Southern California, Arizona and Northern Mexico is a source for the liquid wax ester called jojoba oil. Previous reports on the economic potential [1-4], chemical utilization [5-9] and the acid and alcohol composition [10-16] reflect the industrial importance and unusual chemical nature of jojoba oil. The chemical composition of jojoba oil has been determined by using two procedural improvements which had been applied previously [18] to long chain esters containing erucic acid from *Lunaria biennis* and *Crambe abyssinica*. The chain length distribution of the alkyl and acyl moieties in the wax esters of the two plants is similar. According to earlier [11, 18, 19] reports, the position of unsaturation in jojoba and *Lunaria* alcohols for a given chain length is identical. Certainly by G.C. analysis, the peaks for the alcohols from jojoba and *Lunaria* were completely superimposed and no difference in retention data for the alcohols could be detected [17].

Since it is generally assumed that alcohols and acids in animal tissues are in equilibrium and that acids are produced by the normal "de novo" process [20] further information about long chain alcohols in the vegetable kingdom should be a valuable step towards an understanding of this theory. Because of its commercial interest, it
was felt desirable to examine the monounsaturated alcohols of jojoba oil — and to determine the position of the double bond in the major homologues.

II. Experimental

A. Saponification of jojoba oil

Jojoba oil (10 g) was refluxed for 8 hr in anhydrous ethanol (156 cm$^3$) containing 5% hydrochloric acid (7 cm$^3$) and anhydrous benzene (12 cm$^3$). After removal of most of the solvent by distillation, the reaction mixture was dissolved in diethyl ether (100 cm$^3$) and washed with water. Finally the diethyl ether was removed under vacuum and the resulting ethyl esters and long chain alcohols (9.90 g) were recovered.

The product of ethanolysis (9.42 g) was saponified by refluxing overnight in 1.0 M methanolic KOH (40 cm$^3$) containing water (2.0 cm$^3$). The hydrolysis mixture was extracted with diethyl ether and the ether extract washed with distilled water.

The ether extract was dried over sodium sulphate and the ether removed under vacuum to yield the unsaponifiable matter (4.88 g). The acids (4.54 g) were recovered from the basic hydrolysis solution after acidification and ether extraction.

B. Isolation of jojoba alcohols

The unsaponifiable matter (6.0 g) in pyridine (180 cm$^3$) and acetic anhydride (60 cm$^3$) was stirred overnight. The reaction mixture was added to water (420 cm$^3$) and extracted with ether (3 × 120 cm$^3$). The ether extracts were washed with water, dried over sodium sulphate and the solvent removed by distillation. The resultant mixture was then chromatographed on silica gel (50 g Merck, 0.2—0.5 mm particle size) when the alkyl acetates were eluted with 10% ether in light petroleum (40—60°C).

C. Gas chromatography

The alkyl acetates were separated by chain length on a 20% Apiezon L on Phase Sep N (44—60 mesh) column (7 ft × $\frac{1}{4}$ in) in a Pye model 105 instrument. Collection of fractions up to chain length $C_{20}$ was achieved isothermally at 250°C and the remaining fractions were collected on temperature programming to 280°C.

Analytical G.C. was performed on a 1% Apiezon L column on Gas Chrom Z (80—100 mesh) in a Pye 104 dual column instrument.
D. Thin layer chromatography

Individual fractions from the preparative scale G.C. separation (20 mg) were chromatographed on 16.7% AgNO₃—silica gel thin layer plates using petroleum ether; diethyl ether (90 : 10) as an eluting solvent.

E. Von Rudloff oxidant

The stock oxidant solution was prepared from sodium periodate (0.2 M) and potassium permanganate (0.005 M).

The purified C₁₈ acetate (4 mg) was dissolved in purified t-butanol (1 cm³) and stock oxidant solution (2 cm³) and the reaction mixture shaken for 1 hr.

F. Analysis of oxidant products

An aliquot (20 µl) and injected directly on to a Poropak Q (80–100 mesh) column (18 in × ½ in) at 130°C. When the solvent, acetic acid and propionic acid had been eluted at this temperature the column temperature was raised to 200°C at a rate of 6°C per min to permit the elution of butanoic and pentanoic acids. Elution of hexanoic, heptanoic and octanoic acids was completed isothermally at 200°C and finally the temperature raised to 230°C at 6°C per min to elute nonanoic acid.

The remainder of the von Rudloff oxidation mixture was extracted with diethyl ether (4 × 10 cm³) and methylated with diazomethane. The methylated products were separated by T.L.C. into monomethyl esters and half acetylated methyl esters each of which was further analysed by G.C. on an Apiezon L column.

III. Results and discussion

Jojoba wax ester was hydrolysed by the method previously reported [17]. Comparison of the non-saponifiable matter with unreacted wax ester, octadecyl stearate, eicosane and octadecanol by T.L.C. indicated the presence of a small amount of polar material which has not been further examined.

By extraction of several T.L.C. plates it could be shown that a small quantity of wax ester remained unchanged.

After conversion to the acetates, the alcohols were separated from the other unsaponifiables by column chromatography. The alkyl acetates were analysed on a 1% Apiezon L column and the results of area measurement show good agreement with previous analysis [13]. Then the acetates were separated into their individual homologues by preparative G.C. on Apiezon L. The C₂₀ and C₂₂ alkyl acetates were better than 99% pure by analytical G.C. and by AgNO₃ T.L.C. The C₁₈ and C₂₄ alkyl acetates required further purification on AgNO₃—T.L.C. to remove the saturated homologues.
Table 1
Composition and isomer distribution of the monounsaturated alkyl moieties in wax esters from jojoba oil.

<table>
<thead>
<tr>
<th>Chain lengths of alkyl moiety</th>
<th>% composition</th>
<th>Position of double bond (% composition)</th>
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</thead>
<tbody>
<tr>
<td>18:1</td>
<td>1.0</td>
<td>ω9 69 ω8 3 ω7 16 ω6 12 ω5 27</td>
</tr>
<tr>
<td>20:1</td>
<td>48.7</td>
<td>ω9 83 ω8 11 ω7 4 ω6 2 ω5 27</td>
</tr>
<tr>
<td>22:1</td>
<td>4.9</td>
<td>ω9 89 ω8 4 ω7 5 ω6 2 ω5 27</td>
</tr>
<tr>
<td>24:1</td>
<td>6.4</td>
<td>ω9 63 ω8 2 ω7 3 ω6 5 ω5 27</td>
</tr>
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</table>

The position of the double bond in each of these unsaturated alcohols was determined by the von Rudloff oxidation method. In order to avoid the labour and inexactitudes of extraction of the short chain fatty acids from aqueous solution, the procedure previously developed by us for Sperm Whale Oil was adopted [21].

Direct injection of the oxidation mixture into a Poropak Q column permitted the analysis of all free acids C2-C10. In addition to the determination of the monobasic acids which are derived from the ω end of the molecule, the difunctional acetoxy methyl esters were extracted and analysed on an Apiezon L column, confirming the results of free acid analysis.

Earlier work on unsaturated acids and alcohols of jojoba wax [11] claimed that the position of the unsaturation was ω9 viz eicos-11-enol and eicos-11-enoic acid. Many comparisons have been made between the acids and alcohols of jojoba and those of Lunaria seed oil. Recently one of us [18] analysed the monobasic acids obtained after oxidation of the Lunaria monoenoic acid and showed their composition to be in the ratio 2 : 1 : 3 : 94/hexanoic : heptanoic : octanoic : nonanoic acid. The present work is the first attempt to separate each homologue in a pure form and then determine the position of unsaturation.

Our results (table 1) indicate that although the ω9 position is the major double bond position, it is by no means the only one. Thus tetracosenol has 27% of the ω4 isomer whilst octadecenol has significant proportions of the ω5 (12%) and (16%).

The absence of monobasic acids of the C4, C5 and C6 chain length in the oxidation of the acids of jojoba wax in earlier work makes it seem unlikely that Schlenk’s [20] concept of rapid equilibrium between acids and alcohols which holds in fish, applies in jojoba seeds.

References

[1] N.T. Mirov, Chemurg. Dig. 9 (7) (1950)