Technical

Jojoba Oil Analysis by High Pressure Liquid Chromatography and Gas Chromatography/Mass Spectrometry[1]

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ABSTRACT AND SUMMARY

Two analytical procedures for determining compositions of jojoba liquid wax esters are described and compared. One, the more tedious, involves separation of wax ester homologs by high pressure liquid chromatography followed by determination of the acid and alcohol moieties from each homolog. The second allows rapid determination of wax ester composition by gas chromatographic separation of hydrogenated jojoba wax esters according to chain length, followed immediately by ancillary mass spectrometric identification of the acid and alcohol moieties. Double bonds in the alkyl chains in jojoba liquid waxes were almost exclusively (98%) ω-9, when examined by gas chromatography/mass spectrometry (GC/MS) and ozonolysis/GC/MS.

INTRODUCTION

Increased demand for renewable energy and raw material sources has rekindled interest in jojoba [*Simmondsia chinensis* (Link) Schneider] oil, a liquid wax ester obtained in high (ca. 50-60%) yield from the seeds of a desert shrub native to the arid lands of southwestern North America. A serious effort towards jojoba utilization is underway, evidenced by the creation of the Committee on Jojoba Production Systems Potential of the National Research Council and by the establishment of embryonic plantations in Arizona, California, and Northern Mexico. An earlier committee on Jojoba Utilization of the National Research Council reported (1) that although jojoba has great potential as a new industrial crop, further research is still needed. In the present study, we have applied modern chromatographic and spectrometric techniques to the analysis of jojoba oil to answer the following questions: What combinations of acids and alcohols make up the wax esters? What, if any, positional isomers exist in the component fatty acids and alcohols?

EXPERIMENTAL PROCEDURES

The principal jojoba oil (identified as Apache) investigated in this study was a clear, sediment-free oil, expeller-pressed by Anderson IBEC, Strongsville, OH, from seeds gathered at the San Carlos Apache reservation in Arizona. The other two oils (Hi MW and Lo MW) were hexane-extracted from seeds harvested by H. Nenninger at the San Nicolas plantation near Kino Bay, Sonora, Mexico.

High pressure liquid chromatography (HPLC) was used to separate components according to chain length. Two 12 x 1/4 in. reverse-phase columns (μ-Bondapak C_{18}, Water Assoc., Framingham, MA) were connected in tandem. The solvent systems were acetonitrile-acetone (2:1) for intact oils and acetonitrile (100%) for free alcohols, and components were detected by differential refractometry. Thin layer chromatography (TLC) was on 0.25 mm layers of Silica Gel G in a hexane-ether (80:20) solvent system. Intact oils, whether hydrogenated or not, were analyzed by gas chromatography (GC) on 3 ft x 1/8 in. columns packed with 3% OV-1 on Gas Chrom Q (2). GC analysis of fatty acids (as methyl esters) and alcohols [as trimethylsilyl (TMS) ethers] was carried out on two 4 ft x 1/8 in. columns, one packed with 5% Apiezon L on Chromosorb W and the other with 3% Silar-5 CP on Gas Chrom Q. (All GC packings were obtained from Applied Science Laboratories, Inc., State College PA.) The gas chromatography/mass spectrometry (GC/MS) apparatus and its computerized data acquisition and reduction system have been previously described (3). The GC operating conditions for both analytical GC and GC/MS were optimized for the samples under investigation; for analytical GC, detection was by flame ionization.

Oils and oil fractions (from HPLC) were prepared for analysis of their component fatty acids and alcohols in the following manner. A small (<5 mg) sample was dissolved in a minimum of toluene and 1.2 ml of 5% NaOH in methanol was added. This solution was refluxed for 1½ to 2 hr, cooled, and 1-2 ml of 10% BF_3 in methanol was added. After this solution had been further refluxed for 1/2 hr, it was cooled and transferred to a separatory funnel by rinsing with ether (ca. 5 ml) and water (ca. 20 ml). The methyl esters and free alcohols in the organic solvent phase were recovered by ether extraction (4 x 5 ml). The ether was removed on a steam bath under nitrogen, and the methyl esters and alcohols were separated by preparative TLC on Silica Gel G. The methyl esters were analyzed directly by GC but the alcohols were converted to ethers with bis-(trimethylsilyl)-trifluoroacetamide before GC analysis. A scaled-up TLC preparation (ca. 100 mg) was made to obtain alcohols for subsequent separation by HPLC according to chain length.

Double bond positions in the fatty acids (as methyl esters) were determined by GC/MS of their methoxy derivatives (4). Ozonolysis/GC (5) was used to establish double bond positions in the isolated alcohols, as TMS ethers. These ethers were also identified by GC/MS as well as by GC retention characteristics. As a test for analytical accuracy, a sample of mixed eicosenols prepared from reduction of *Limnanthes* triglyceride oil was analyzed by ozonolysis/GC/MS. This mixed sample, which contained only the Δ4, Δ5, and Δ6 isomers (some double bond migration occurred during reduction) in a ratio of 10:80:10, was added in small quantity (1/20) to the

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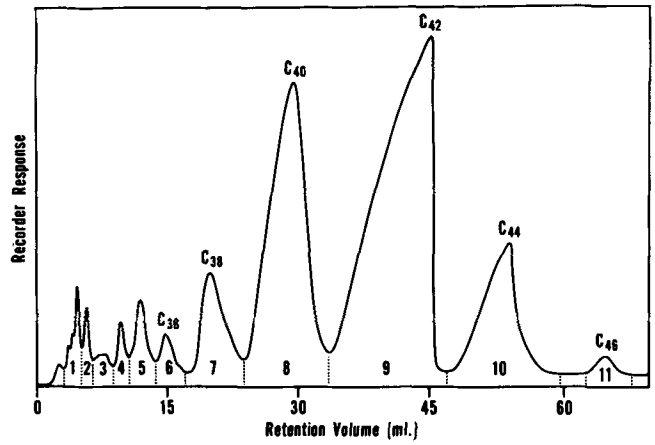

FIG. 1. High pressure liquid chromatography of Apache Jojoba oil. (μ-Bondapak C_{18}, two 12 x 1/4 in. in tandem; acetonitrile/acetone = 2/1.)

eicosenol isolated from the Apache jojoba oil. This mixture, composed of 0.5% Δ4, 4% Δ5, and 0.5% Δ6 plus the jojoba eicosenol, was analyzed by ozonolysis/GC/MS.

For the rapid analytical procedure using only GC/MS, oils were hydrogenated in toluene-ethanol (1:10) with palladium-on-charcoal as the catalyst.

RESULTS AND DISCUSSION

Wax Ester Composition

Prior to Miwa's report (6), jojoba wax esters were assumed to be made up of random combinations of alcohols and acids. He showed that the proportion of C_{42} was far greater than could be expected from random combinations. However, the detailed composition of the wax esters remained unknown because it was virtually impossible to separate large amounts according to chain length. Reverse phase chromatography has long been used to separate homologs but has generally been restricted to shorter chain components than those found in jojoba oil.

However, HPLC on new microparticulated reverse phase packings has now made possible the rapid separation of long chain triglycerides and wax esters by chain length and degree of unsaturation (7). Since the acids and alcohols of jojoba oil are virtually all monoenes, each peak in the chromatogram of jojoba oil (Fig. 1) contained the combinations of ester and alcohol of one chain length.

Fractions 1-5 were composed of polar materials—free alcohols and acids, sterols, and oxygenated compounds, but their identifications are not reported here.

Fractions 6-11, when analyzed by GC, were found to contain the C_{36}, C_{38}, C_{40}, C_{42}, C_{44}, and C_{46} wax esters, respectively. A portion of fractions 7-10 was saponified individually, the acids and alcohols were separated, and each separate fraction was analyzed by GC. The wax ester combinations of each chain length were calculated by adding the area percentages of the corresponding acid-alcohol pairs making up that chain length and dividing by two. For example, the proportion of C_{42} composed of C_{20} acid and C_{22} alcohol = (area % C_{20} acid + area % C_{22} alcohol)/2. These area percentages (acid to corresponding alcohol) were generally in excellent agreement (±3%). The results for the Apache oil are given in Table I.

While the above method is accurate, it is time consuming, requires meticulous attention to detail, and is not suitable to extensive seed screening investigations. Comparable information can be obtained by GC/MS as adapted from the work of Aasen et al. (8). In analysis of standard wax ester mixtures, they found that by summing the ion intensities of three significant ions from each acid-alcohol combination, they could accurately estimate the proportions of the combinations. From the general wax ester formula RCO_2R' where R and R' are the alkyl moieties of

TABLE I

Isomer Composition for Each Chain Length in Jojoba Wax Esters

Wax ester chain length	Alcohol/acid combination	HPLC[a], Sapon.[b]	GC/MS (%)[c]		
		Apache (%)	Apache	Hi-MW	Lo-MW
36	16-20	–	–	–	10
	18-18	–	5	–	15
	20-16	–	95	–	75
38	16-22	4	3	1	3
	18-20	21	14	14	18
	20-18	70	80	78	78
	22-16	5	3	7	1
40	16-24	1	1	2	1
	18-22	4	5	5	5
	20-20	82	81	70	85
	22-18	12	12	21	8
	24-16	1	1	2	1
42	18-24	2	3	2	3
	20-22	21	21	16	20
	22-20	75	74	80	76
	24-18	2	2	2	1
44	18-26	–	–	1.5	–
	20-24	12	9	9	7
	22-22	28	21	21	19
	24-20	60	70	68	74
	26-18	–	–	0.5	–
46	20-26	–	–	2	–
	22-24	–	–	20	–
	24-22	–	–	63	–
	26-20	–	–	15	–
48	22-26	–	–	3	–
	24-24	–	–	61	–
	26-22	–	–	36	–

[a]HPLC = High pressure liquid chromatography

[b]HPLC separation and saponification, followed by separate analysis of alcohols and acids.

[c]GC/MS = Gas chromatography/mass spectrometry.

TABLE II

Composition of Jojoba Oil Samples
Percentage by Gas Chromatography

Wax ester chain length	Apache	Hi-MW	Lo-MW
34	0.1	0.1	0.2
36	2	0.4	2
38	7	1	9
40	30	9	42
42	50	56	41
44	10	24	5
46	0.8	7	0.3
48	0.1	2	trace
50	–	0.1	–

the acyl and alcohol groups, respectively, the intensities of the ion RCO_2H^+, $RCO_2H_2^+$, and $(R'\text{-}1)^+$ were summed for each combination possible within a given chain length. The relative percentage of each sum then represents the relative percentage of that particular combination. Since our mass spectra were taken in a repetitively scanning mode throughout the GC run, we summed (by computer) the appropriate ion intensities across each GC peak. Results of this analysis are also given in Table I. This GC/MS method can be applied only to fully saturated wax esters (8) and so we completely hydrogenated the oils before analysis. Although unsaturation cannot be determined by this procedure, the wax ester composition and the constituent alcohol and acid compositions can be determined by a single GC/MS operation. The wax ester isomer compositions of two other oils were determined by GC/MS (Table I). These oils had been found by preliminary GC analysis to contain unusually large proportions of low (Lo MW) and high (Hi MW) molecular weight wax esters (Table II). Results from the GC analysis of their derivatized acids and alcohols are compared with those of the Apache oil in Table III.

Double Bond Position

Jojoba oil wax esters were assumed to be ω9-unsaturated (6) until a recent report indicated that several isomeric fatty alcohols were present (9). After a sample of the Apache oil had been saponified and the methyl esters (derived from the fatty acids) and alcohols separated, methoxy derivatives were formed and subjected to GC/MS. The derivatives of the methyl esters showed only small amounts of positional isomers other than ω9, and only in the 16:1 (0.1% ω7) and 18:1 (1% ω7). This technique, however, did not give clear-cut fragmentation at the methoxyl group when applied to the alcohols. To solve the problem, the alcohols were separated by chain length with HPLC and the ozonolysis/GC/MS procedure was used to establish the sites of unsaturation. By this technique, only ω9 unsaturation was found. To check the detection limits, the mixture of isomeric C_{20} alcohols was prepared and analyzed, as described above. The Δ4 and Δ6 isomers were readily detected at even the 0.5% level. We have therefore concluded that the jojoba alcohols are exclusively ω9-unsaturated.

TABLE III

Composition of Fatty Alcohols and Fatty Acids from Jojoba Oils
(Percentage by Gas Liquid Chromatography Area Integration)

Chain length: No. of double bonds	Fatty alcohols			Fatty acids		
	Apache	Hi-MW	Lo-MW	Apache	Hi-MW	Lo-MW
14:0	tr[a]	tr	tr	tr	0.1	0.1
16:0	0.1	0.4	0.5	1.2	1.2	1.8
16:1	–	tr	tr	0.3	0.4	0.5
18:0	0.2	0.2	0.2	0.1	0.1	0.2
18:1	1.1	2.0	2.1	11	5.2	12
20:0	tr	2.1	3.5	0.1	0.1	0.1
20:1	44	15	52	71	68	74
22:0	1.0	3.2	1.5	0.2	0.9	0.5
22:1	45	49	35	14	18	9.2
24:0	–	0.9	0.5	tr	0.5	tr
24:1	8.9	23	3.9	1.3	4.8	1.1
26:0	–	0.6	tr	–	0.1	tr
26:1	–	3.3	0.3	–	0.4	tr

[a]"tr" denotes 0.01-0.05%. Traces of odd-numbered chain components and some polyenoic C_{18} and C_{20} acids were also detected.

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