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Jojoba Oil Wax Esters and Derived Fatty Acids and Alcohols: Gas Chromatographic Analyses[1]

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ABSTRACT

HCl-catalyzed ethanolysis followed by saponification readily surmounts the resistance of long chain wax esters to direct hydrolysis by alkali. Additionally, choosing ethyl instead of methyl esters allows baseline separations between long-chain alcohols and corresponding esters in gas liquid chromatographic (GLC) analysis of total alcohol and acid components before saponification. Liquid wax esters were analyzed on a temperature-programmed 3% OV-1 silicone column. Geographical and genetic effects on the variability of jojoba oil composition were investigated with five different seed samples. Major constituents in jojoba seed oil from shrubs in the Arizona deserts, as indicated by GLC analyses of oil, ethanolysis product, isolated fatty alcohols and methyl esters of isolated fatty acids, were C_{40} wax ester 30%, C_{42} wax ester 50% and C_{44} wax ester 10%; octadecenoic acid 6%; eicosenoic acid 35%, docosenoic acid 7%, eicosenol 22%, docosenol 21% and tetracosenol 4%. Oil from smaller leaved prostrate plants growing along California's oceanside showed a slight tendency toward higher molecular size than oils from the California desert and Arizona specimens. The wax esters are made up of a disproportionately large amount of docosenyl eicosenoate and are not a random combination of constituent acids and alcohols. *Lunaria annua* synthetic wax ester oil was used as a model for evaluating the analytical procedures.

INTRODUCTION

Jojoba (*Simmondsia californica*) oil is a liquid wax ester mixture extracted from the seeds of a desert shrub native to Arizona, California and northern Mexico. Although the economic potential (1-4) and chemical utilization (5-9) of jojoba oil have been reported intermittently, its wax ester composition has never been investigated and its acid-alcohol composition has not been redetermined since early investigations on the oil in 1933 (10) and in 1936 (11,12). McKinney and Jamieson (12) identified the acids and alcohols by melting points, saponification values, neutralization values, iodine values, acetyl values and refractive indices of fractions that were isolated by distillation of ester derivatives under reduced pressure. Crystallographic spacing was also used to identify the major acid. The acid-alcohol composition has been quoted frequently (2,3,6,9,13-16) ever since the 1936 reports without any confirmation because of the challenges of high resistance of the oil to saponification and isolation as pure fractions and because of the lack of convenient and reliable quantitative means of analysis.

This report, made possible by two procedural improvements, follows our earlier work on erucic acid-containing long chain wax esters (16,17). The first improvement was a gas liquid chromatographic (GLC) procedure that gives complete resolution of the high-molecular-weight wax esters; the second, a two-step procedure that completely hydrolyzes the wax esters to fatty acids and alcohols.

An expanded procedure of saponification was necessary to convert the wax esters totally to free fatty acids and alcohols because ethanolic KOH saponification or alkoxide reaction did not lyse the wax completely even under rigorous conditions, e.g., 1.00 M anhydrous ethanolic sodium ethoxide as catalyst and reflux for seven days. Successful conversion to ethyl esters and free alcohols was achieved with 5% HCl in anhydrous ethanol. The ethyl esters were readily saponified, and the free acids and alcohols were recovered conventionally.

GLC analysis of the acids and alcohols showed appreciable differences in composition from that reported by McKinney and Jamieson (12). The wax esters of jojoba were not a random combination of constituent acids and alcohols but instead were partially specific for docosenyl eicosenoate. Slight variations in oil composition were observed among seed samples of different genetic strain or growth environment.

Lunaria annua synthetic wax ester oil (17) served as a model for evaluating ethanolysis, saponification, identification and quantification procedures.

EXPERIMENTAL PROCEDURES

Jojoba Seed

Quantitative procedures were developed by use of a seed sample collected in the Arizona deserts by Boyce Thompson Institute, Superior, Arizona. Consistency in composition for seeds from similar geographical environments was tested with another sample collected from the Tucson suburbs by H. Muramoto of the University of Arizona. Seeds from the same phenotype species adapted to the hot and dry California desert were provided by D.M. Yermanos of the University of California, Riverside. A different phenotype growing along the southernmost coast of California, which is prostrate and has smaller leaves and seeds than the Arizona-type, was also harvested by Professor Yermanos. A sample of undisclosed origin was provided by C.Y. Hopkins of the Canadian National Research Council, Ottawa.

Jojoba Oil Extraction

Jojoba seeds were ground in a 6 in. hammer mill and placed in a Soxhlet extractor for reflux with petroleum ether solvent (pb 33-57 C). After 24 hr, the solvent was removed from the extract by heating and evaporating under a stream of nitrogen. Yields of nonvolatile oil ranged from 40.3% to 52.1% of the ground meal. An additional 24 hr extraction of the meal increased the yield only 0.1% to 0.4%. Composition of the oil from the second extraction was always identical to the first when the four different seed accessions were compared.

Ethanolysis of Wax Esters

An estimated 1 mmole of jojoba oil was refluxed with

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10.0 g of 5.0% HCl in anhydrous ethanol. Anhydrous benzene (1.2 ml) was added to increase the solubility of the oil in ethanol. Although solubilization of the oil occurred in 15 min, the system was refluxed for 8 hr. The solvent and HCl were removed by distillation, replenishing the reaction vessel three times with ethanol. The products were dissolved in ethyl ether and washed with water before drying in the vacuum oven at 45 C. Recoveries of alcohol-ester product indicated a consistent 99% conversion, e.g., 0.6821 g recovered from 0.6432 g of Arizona desert jojoba oil.

Isolation of Free Acid and Alcohol Fractions

Half of the ethanolysis product was saponified at reflux overnight in 10.0 ml of 1.0 M KOH in ethanol with 1.0 ml of water added. The hydrolyzate was thoroughly extracted with ethyl ether and the extract, which contained alcohols, was washed with 1.0 M aqueous KOH and water. The combined aqueous phase and washings were acidified and extracted with ethyl ether and the extract, which contained the acids, was rinsed with water and dried. For example, 0.1702 g of alcohols and 0.1730 g of acids were recovered from 0.3590 g of ethanolysis product. With the allowance for change in weight from ethyl ester to free acid, recovery was 100% of theory based on an average molecular weight of 606 calculated for this jojoba oil.

Gas Liquid Chromatography

The ethanolysis products, diazomethane-treated fatty acids and alcohols from jojoba and *Lunaria* wax esters were analyzed on nonpolar Apiezon L and Resoflex 446 (LAC-2R 446) polyester columns. Except for detailed fractionation of the unsaturated esters and alcohols, results from Apiezon L were taken for assignment of composition. The Resoflex 446 column showed a decrease in quantitative response for higher homologs, especially the alcohols. The alcohols were chromatographed without acetylation or silylation because, with the Apiezon L column, results from the model *Lunaria* alcohol sample were as quantitative as results from its silylated derivative. Ethanolysis products from *Lunaria* synthetic wax ester also gave a molar response for the alcohols as quantitative as for the ethyl esters. Additionally, jojoba alcohols were cochromatographed with *Lunaria* alcohols to confirm the identity of jojoba alcohols and to compare the composition of the admixture (1:1) with the calculated combined composition.

After the identification of components by their equivalent chain lengths (ECL) (18,19) at 230 C, the Apiezon L column was heated with a variety of multilinear programs to attain the most efficient quantification for each sample. Each figure in this report is shown with its program along the abscissa. Other conditions were as follows:

Apiezon L. Twenty per cent on Chromosorb W, acid-washed, silylated, 100-120 mesh; stainless steel column, 0.2 cm i.d., 0.3 cm o.d., 100 cm long; helium carrier gas, 40 ml/min, 50 psig; injection port 280 C, on-column; flame ionization detector oven 360 C; F&M 810 gas chromatograph.

Resoflex 446. Five per cent on Gas Chrom Q, acid-washed, silylated, 60-80 mesh; glass-coiled column, 0.6 cm o.d., 366 cm long; helium carrier gas, 50 ml/min, 30 psig; injection port 250 C, on-column; flame ionization detector oven 275 C; Packard 7401 gas chromatograph.

Wax esters of jojoba and *Lunaria* were analyzed on the high temperature silicone liquid phase OV-1 under isothermal and temperature-programmed conditions. Equipment and optimized experimental conditions were as follows:

OV-1. Three per cent on Gas Chrom Q, acid-washed, silylated, 100-120 mesh; stainless steel column, 0.2 cm i.d., 0.3 cm o.d., 100 cm long; helium carrier gas, 100 ml/min, 100 psig; sample sizes 0.01 to 1.0 μl, diluted tenfold in toluene; programming rate 2 or 4 C/min from 250 to 350 C; injection port 350 C, on-column; flame ionization detector oven 385 C; F&M 810 gas chromatograph.

Peaks were integrated by the Infotronics CRS-40 system with playback settings adjusted to high sensitivity and threshold levels down to 0-2 μV. Digital electronic integration gave the same results as the "cut and weigh" method.

RESULTS AND DISCUSSION

L. annua Synthetic Wax Ester Model

GLC analysis of products from *Lunaria* as a model is given in Table I for ethanolysis of wax esters, for relative detector response of acids vs. alcohols and for agreement between calculated and experimentally found wax ester compositions. No unreacted wax esters were detectable (by gas chromatography) in the ethanolysis product, the recovery of which was 96.3% of theory. The slight shift in

TABLE I

Lunaria Synthetic Liquid Wax Ester as Model for Ethanolysis, for GLC Detector Response and for Randomness in the Combination of Acids and Alcohols

Number of carbon atoms, double bonds, and double bond positions	Mole per cent after ethanolysis: Acid	Alcohol	Number of carbon atoms in wax ester	Randomly combined, converted to weight %	Found by GLC, area %
14:0	0.04	0.04	32	0.1	0.1
16:0	0.9	0.8	34	1	1
16:1	0.1	0.1	36	9	10
18:0	0.1	1	38	2	2
$18:1^{9}$	13	12	40	28	29
$18:2^{9,12}$	3	3	42	15	15
$18:3^{9,12,15}$	0.2	0.2	44	20	20
20:0	0.3	0.4	46	20	19
20:1	0.4	0.2	48	5	4
22:0	0.1	2			
$22:1^{13}$	22	19			
24:0	0.2	2			
$24:1^{15}$	10	9			
Total	$50._{34}$	$49._{74}$			
(Relative detector response)	(1.00)	(0.99)	MWn	616	614

composition from unsaturated to saturated alcohols, when contrasted with the acids, indicates the extent of ethylenic bond reduction that occurred in the fatty acids during sodium reduction (20) of the glyceride linkages in *Lunaria* oil. This partial reduction was not detected during our earlier investigation (17) because of limited resolution by the GLC columns.

The excellent agreements between total acid and alcohol responses and between calculated randomly combined and actual GLC compositions of wax esters raised the level of confidence in the quantitative aspects of this latest investigation. Calculations were made by equating area per cent to weight per cent. In the quantification of alcohols, the ideal situation of having a known internal standard mixture that approximates the composition of the unknown sample but does not cause overlapping of peaks was adequately fulfilled by the ethyl esters derived from *Lunaria* wax esters. The fatty acid composition of *Lunaria* triglyceride oil, as extracted from seed, was determined earlier by GLC analysis of methyl esters (17,21) and reconfirmed in this investigation by GLC analysis of the ethanolysis product from *Lunaria* triglyceride oil.

Jojoba Fatty Acids and Fatty Alcohols

The composition of jojoba acids and alcohols is listed in Table II. Values for the GLC analyses were averages from five optimized runs on the Arizona desert sample. The iodine value of the oil calculated from this acid-alcohol composition is 82.2 and is in excellent agreement with the experimental value, 82.1. No unreacted wax esters were detected by GLC analysis of the ethanolysis product on the OV-1 column. The weight ratio of acid to alcohol was essentially 50:50, when calculated from either the ethanolysis product or the isolated acid and alcohol fractions.

Unlike the results in the report by McKinney and Jamieson (12) (Table II), a significant amount of oleic acid was found in our jojoba oils, illustrated by the methyl oleate peak with ECL 18.4 in Figure 1A. Eicosenoic acid (ECL 20.4) represented 70% of the total acids, whereas erucic acid (ECL 22.4) was only half as much as reported by them. They did not identify any tetracosenoic acid (ECL 24.4) in their higher boiling fractions.

More than 90% of the alcohols were represented by eicosenol and docosenol in equal amounts (Table II and Fig. 1B). Tetracosenol (ECL 23.6, saturated *n*-alcohols as

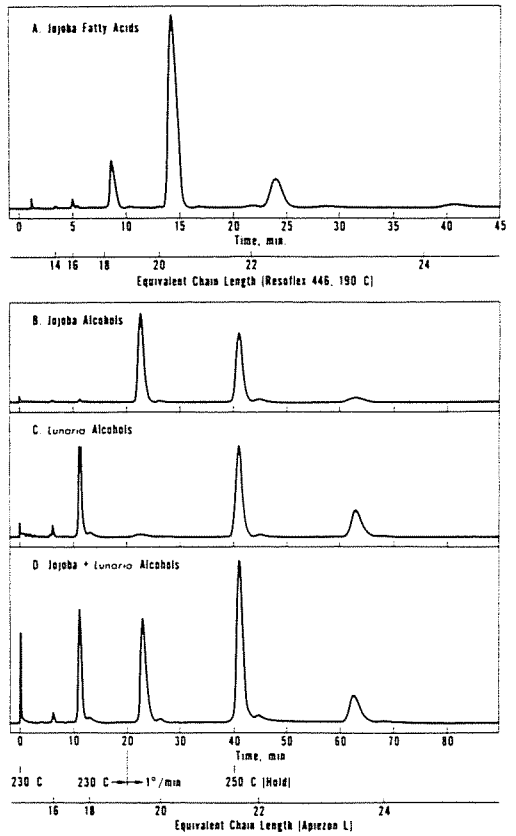

FIG. 1. (A) Fatty acid composition of jojoba oil after HCl-ethanolysis, saponification, acidification and diazomethylation. (B) Fatty alcohol composition of jojoba oil after HCl-ethanolysis, saponification and ethyl ether extraction. (C) Fatty alcohol composition of *Lunaria annua* synthetic liquid wax ester after same treatment as B. (D) Admixture of jojoba and *Lunaria* alcohols in 1:1 portions.

references) was present in appreciable quantity although it was not identified in earlier studies of jojoba oil. The solid alcohol fraction recovered by McKinney and Jamieson, and presumed to be hexacosenol, may have been mostly tetracosenol. Their experimental acetyl value, 118.2 for this

TABLE II

Composition of Jojoba Acids and Alcohols[a]

Number of carbon atoms and double bonds	By ethanolysis and GLC, wt %[b]		By fractionation and GLC, wt %[c]		McKinney and Jamieson (12), wt %	
	Acids	Alcohols	Acids	Alcohols	Acids	Alcohols
14:0	0.1	0.0	0.1	0.0		
16:0	0.9	0.3	0.8	0.1	1.64[d]	
16:1	0.3	0.0	0.2	0.0	0.24	
18:1	6	0.7	6	0.6	0.66	
18:2	0.1	0.0	0.1	0.0		
20:0	0.1	1	0.0	0.4		
20:1	35	22	35	23	30.30	14.60
20:2	0.1	0.0	0.1	0.0		
22:0	0.2	1	0.0	0.5		
22:1	7	21	7	21	14.20	33.70
22:2	0.1	0.0	0.1	0.0		
24:0	0.0	0.0	0.0	0.1		
24:1	0.5	4	1	4		
Total	$50._4$	$50._0$	$50._4$	$49._7$	47.04	48.30

[a]From seed oil of Arizona desert sample.
[b]Calculated after ethyl esters were corrected to free acids.
[c]Calculated as if both fractions were remixed as free acids and alcohols.
[d]Reported as total saturated acids.

fraction, corresponds to triacontenol instead of hexacosenol, which correctly has a calculated acetyl value of 132.7 rather than 117.3 as reported. In the present study no C_{26} acid or alcohol was detected by GLC analysis, even when a large sample was injected and rapidly programmed on the OV-1 column.

No dienyl alcohols were found when the isolated alcohols were analyzed on the polyester column, On the other hand, small amounts of the saturated eicosanol and docosanol were detected, whereas McKinney and Jamieson reported no saturated alcohols by the less sensitive bromination-fractional distillation method or the cold concentrated sulfuric acid treatment. Rapid GLC scans in the free alcohol region of the total oil showed small amounts of components corresponding to free eicosenol (0.3%), docosenol (0.5%) and tetracosenol (0.2%). No attempt was made to detect free fatty acids because the acid value was 0.0. At high sensitivity (x/256) the oil sample showed 15 unknown peaks equivalent to C_{33}-C_{70} paraffins that totaled 1.0% of the oil.

Individual jojoba alcohols were identified by comparison

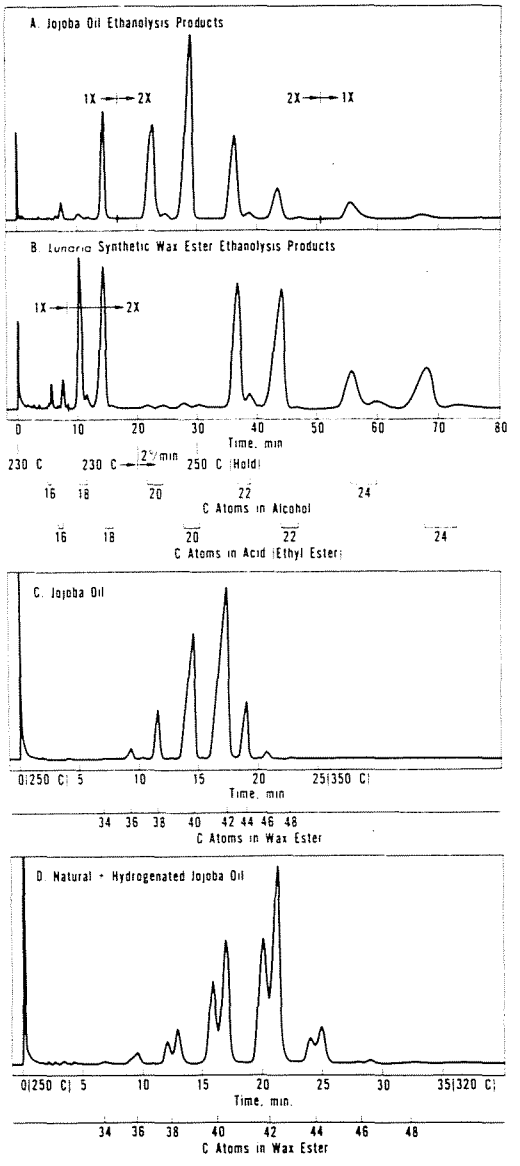

FIG. 2. (A) Ethanolysis product from jojoba oil. (B) Ethanolysis product from *Lunaria annua* synthetic liquid wax ester. (C) Wax ester composition of jojoba oil. (D) Wax ester composition of 2:3 admisture of natural and hydrogenated jojoba oils. (A and B on Apiezon L; C and D on OV-1).

with *Lunaria* alcohols and by admixture in equal quantities of jojoba and *Lunaria* alcohols, as illustrated in Figure 1C and 1D. The composition of the cochromatographed alcohols was identical to that calculated for the sum of the two samples. According to earlier reports (11,17,22), the positions of unsaturation in jojoba and *Lunaria* alcohols for a given chain length are identical. Because peaks for the alcohols from jojoba and *Lunaria* were completely superimposed and no differences in ECL could be detected even when run independently, no steps were taken to substantiate further the identity of the alcohols.

Evidence for the quantitative GLC response of alcohols in relationship to the acids was obtained by comparison of the alcohols with cochromatographed ethyl esters rather than methyl esters. Because the ECL values of ethyl esters on Apiezon L are 0.7 unit greater than methyl esters, baseline separations can be made between saturated alcohols and unsaturated ethyl esters. A single GLC curve provided an excellent breakdown of the acids and alcohols, as illustrated in Figure 2A. Clearly the eicosenoic acid moiety predominates among the jojoba components and the level of oleyl alcohol is substantially lower than those of C_{18} acids. In contrast, the excellent acid-alcohol pairings in the model *Lunaria* ethanolysis product (Fig. 2B) proved that the GLC responses of acids and alcohols were quite similar and that the processes in the production of synthetic liquid wax esters were totally random, as anticipated. The readily identifiable pairings for *Lunaria* in Figure 2B provided the basis for identification of jojoba peaks in Figure 2A.

Jojoba Wax Esters

The composition of jojoba oil by GLC analysis is listed in Table III. The values are averages from five optimized runs on the Arizona desert sample. Fig. 2C resulted from GLC conditions that gave the highest quantitative response for the longer components, while maintaining complete resolution of individual components at a minimum of retention time. Sample size was gradually increased until no change in composition occurred ($\geqslant 0.05$ μl). At low sample sizes ($\leqslant 0.01$ μl) the minor constituents either diminished in percentage markedly or became undetectable. The peaks were identified by cochromatography of the jojoba oil with synthetic *Lunaria* wax esters. The incremental ECL (23) of the carboxyl group in wax esters was determined as +2.3 for OV-1 at 250-350 C; i.e., if a long chain linear paraffin were converted to a saturated wax ester by inserting an oxygen atom into the middle of the chain and oxidizing one of the adjoining methylenes to a carbonyl, the resulting increase in ECL would be 1.3. A similar conversion of the paraffin to a methyl ester would have given a +1.7.

Natural and hydrogenated jojoba oils were admixed at ratios of 1:1, 1:2, 1:3 and 2:3 during the study of relative detector responses between unsaturated and saturated wax esters, and conditions were optimized so that maximum resolution could be attained (Fig. 2D). The unsaturated components were not completely resolved from their saturated counterparts. Nevertheless, when grouped according to chain lenght, the composition of the cochromatographed sample was identical to both the unsaturated and the saturated samples. Further proof of identical quantitative response was obtained when tripalmitin was used as the internal standard (ECL 50.9 with saturated wax esters as references). The relative response of tripalmitin was the same whether mixed with the natural or with the hydrogenated jojoba oil.

The low resolution observed for the C_{36} components in Figure 2D suggests that the saturated component in the curve was the monounsaturated eicosenyl palmitate before hydrogenation. Diunsaturated oleyl oleate, if present, would have the same resolution from the saturated com-

TABLE III

Composition and Nonrandomness of Jojoba Wax Esters[a]

Number of carbon atoms in wax ester	GLC Area %	GLC Mole %	Calculated random combination of acids and alcohols, mole %	Calculated 75% specificity for docosenyl eicosenoate, mole %	Boiled to 420 C under nitrogen, mole %
34	0.2	0.2	0.1	0.1	0.3
36	2	2	2	2	4
38	7	8	8	10	9
40	30	30	40	31	33
42	50	49	37	48	39
44	10	10	11	7	10
46	1	1	2	2	4
48	0.2	0.2	0.1	0.2	0.5

[a]Seed oil from Arizona desert sample.

ponent, as seen with the longer components, or, as was actually found, with the *Lunaria* model (ΔECL = 0.5). A similar low resolution was observed for C_{38} in the *Lunaria* model. The monounsaturated palmito-eruco combination predominated over any polyunsaturated C_{18}-C_{20} combination because *Lunaria* oil is deficient in C_{20} acids.

Nonrandomness in Jojoba Oil Biosynthesis

The amount of C_{42} in jojoba oil is greater than expected from random combination of its acids and alcohols, whereas the amount of C_{40} is less than expected. After a number of calculations, it became apparent that eicosenoic acid and docosenol were the major constituents that contributed to the nonrandom wax ester formation. For example, if 75% of the docosenol were specifically assigned to eicosenoic acid and all the remaining acids and alcohols were randomly esterified, a composition as in Table III would result. This nonrandomness implies that at a certain stage of seed oil development, docosenyl eicosenoate is biosynthesized almost exclusively.

Boiling changed the composition of the oil toward randomness. At 757 mm under nitrogen, the temperature of the oil rose rapidly from ambient to 420 C and then gradually dropped to 398 C, where it remained constant. Upon cooling, the oil increased slightly in viscosity over the unboiled but showed no color change. GLC analysis

TABLE IV

Variability in Jojoba Oil Composition

Analyses	Location of growth: Arizona deserts	Tucson suburbs	California deserts	California oceanside	Unknown
Size of seed, g/1000	466	470	835	450	480
Oil content, %	48.4	40.5	52.5	49.4	46.6
Wijs iodine value	82.1	81.7	81.6	81.8	82.6
Wax esters, area %					
C_{34}	0.2	0.2	0.2	0.1	0.1
C_{36}	2	2	2	2	3
C_{38}	7	7	6	4	10
C_{40}	30	30	28	21	34
C_{42}	50	48	51	55	44
C_{44}	10	11	11	15	8
C_{46}	1	2	2	3	1
C_{48}	0.2	0.3	0.3	0.4	0.1
Acids, methyl ester, area %					
14:0	0.2	0.1	0.1	0.1	0.1
16:0	1	1	1	1	2
16:1	0.4	0.3	0.3	0.3	0.3
18:0	0.1	0.1	0.1	0.1	0.1
18:1	11	11	10	7	14
18:2	0.1	0.2	0.1	0.1	0.1
18:3	0.1	0.3	0.3	0.1	0.2
20:0	0.1	0.2	0.1	0.5	0.1
20:1	70	70	70	69	69
20:2	0.2	0.3	0.1	0.1	0.1
22:0	0.2	0.6	0.5	0.8	0.5
22:1	13	13	15	17	12
22:2	0.2	0.1	0.1	0.1	0.1
24:0	0.1	0.1	0.1	0.2	0.1
24:1	2	2	2	3	1
Alcohols, area %					
16:0	0.2	0.1	0.1	0.1	0.2
18:1	1	1	1	1	2
20:0	0.8	0.6	0.5	2	0.6
20:1	46	46	42	30	50
22:0	2	2	2	6	2
22:1	42	41	47	47	40
24:0	0.2	0.2	0.1	0.4	0.3
24:1	7	8	8	13	6

indicated a decrease in C_{42} and a randomizing tendency (Table III). the *Lunaria* wax ester model, boiling at a steady 380 C, did not change in composition.

Variability in Jojoba Oil Composition

The compositions of jojoba oil from two adjacent regions in Arizona were essentially identical (Table IV), even though one seed sample was acquired five years earlier and had been stored at room temperature. The same phenotype shrubs that have adapted themselves to the hot and dry environment of the California desert have increased markedly in seed size but have changed only slightly in chemical composition of the seed oil. Conversely, the different phenotype growing prostrate along the ocean near San Diego had seeds similar in size to the Arizona type but had a distinct shift in oil composition toward larger molecular size. The seed sample with an unknown history on growth environment showed a shift toward shorter chain lengths. This shift is corroborated by the slight increase in iodine value of the oil. Significantly, throughout the five seed samples, the percentage of the major component, eicosenoic acid, remained unchanged at 35% of the total jojoba oil.

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J.W. Hagemann collaborated in the early stages of this investigation; C.W. Blessin recommended use of toluene as GLC diluent for wax ester samples; J.L. Robertson and G.B. Rose made the jojoba oil extractions; D.M. Palmer ran iodine values; F.R. Earle supplied botanical information on jojoba; and Q. Jones, C.Y. Hopkins, D.M. Yermanos and H. Muramoto supplied seed samples.

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