18. Keane, K. W., G. A. Jacobsen, and C. H. Krieger, J. Nutrition *68*, 57 (1959).
19. Rice, E. E., W. D. Warner, P. S. Mone, and C. E. Poling, *Ibid.*, *61*, 253–266 (1957).
20. Rice, E. E., C. E. Poling, P. E. Mone, and W. D. Warner, JAOCS *37*, 607 (1960).
21. Kaunitz, H., and C. A. Slanetz, Proc. Soc. Exptl. Biol. Med., *75*, 322–326 (1950).
22. Kaunitz, H., and R. E. Johnson, J. Nutrition, S. S., 577 (1955).
23. Andrews, J. S., W. H. Griffith, J. F. Mead, and R. A. Stein, *Ibid.*, *70*, 199 (1960).

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Fatty Acids, Fatty Alcohols, and Wax Esters from *Limnanthes douglasii* (Meadowfoam) Seed Oil

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Abstract

Limanathes douglasii seed oil glycerides contain fatty acids which predominantly (97%) have 20 or more carbon atoms. Fatty acids were prepared by saponification; fatty alcohols, by sodium reduction of the glycerides; and liquid wax esters, by *p*-toluenesulfonic acid-catalyzed reaction of the fatty acids with the fatty alcohols. Solid waxes were prepared by hydrogenation of the glyceride oil and of the wax esters. Chemical and physical constants were determined for *Limnanthes douglasii* seed oil and its derivatives. The liquid wax esters had properties very similar to those of jojoba *(Simmondsia chinensis)* seed oil. The solid hydrogenated wax ester was identical in physical appearance and melting point to hydrogenated jojoba seed oil.

Introduction

Limnanthes douglasii (meadowfoam) (3) is a hardy annual herb native to California and adjoining Pacific Coast States. It is grown as a garden ornamental and has fragrant flowers, about 1 in. across, with white or roseate petals, which are yellowish toward the base. Its seeds are 2–2.5 mm in diameter and 3–5 mm long. The first chemical study (5) of the seeds showed that the extracted oil contained a large amount of long-chain fatty acids, 94% of which are longer than C_{18}. The isolation and chemical characterization of the major C_{20} and C_{22} acids also have been reported earlier (2,13), as well as the amino acid composition of the seed meal (14). These investigations were made as part of a research program to discover new crops that have agricultural and industrial potentials (16).

The present study reports selected chemical and physical properties of oil, fatty acids, fatty alcohols, and wax esters derived from seeds of *Limnanthes douglasii*. Because of their similarity a comparison of chemical composition and physical properties between the wax esters and jojoba oil (4,9) is also presented. Jojoba oil is a liquid ester wax of long-chain fatty acids and fatty alcohols rather than a glyceride fat and is potentially a useful raw material for the chemical and allied industries in such fields as plastics, lubricants, pharmaceuticals, and cosmetics (6,8).

Materials

Limnanthes douglasii seed was obtained from Harry Saier of Diamondale, Michigan. Botanical identity was verified by botanists of the Crops Research Division, USDA, Washington, D.C. The jojoba oil was extracted from *Simmondsia chinensis* nuts acquired from Boyce Thompson Institute, Superior, Arizona.

The bp range of the petroleum ether solvent was 33–57C. Purified ethanol was prepared by refluxing bulk, 95% ethanol with ACS-grade potassium hydroxide, 5 g per liter, for 4 hr, and distilled through a column of glass helices; bp 78C at 760 mm. Ethyl ether, toluene, and xylene were ACS grade. Methyl isobutyl carbinol (4-methyl-3-pentanol) was distilled through a column of glass helices; bp 130–132C at 760 mm.

Preparation of Samples

Oil. Oil was obtained by 60 hr Soxhlet extraction with petroleum ether of 0.5 and 1.0 kg. seed samples, which had been ground in a 6 in. hammer mill. The solvent was removed by bubbling through the solution a stream of nitrogen under reduced pressure (ca. 20 mm) and elevated temperature (heating mantle at ca. 80C) until no change in weight was observed.

Fatty Acids. The triglycerides were saponified by calculating the average molecular weight of the fatty acids from the saponification value of the oil (5) and adding 2 meq of potassium hydroxide and 1 meq of water for every meq of fatty acid. Fifty g of oil was saponified in 300 ml of purified ethanol. After refluxing for 2 hr, 1 l. of water was added, and the unsaponifiables were extracted with ethyl ether. The ether extract was washed with 1*N* K_2CO_3 and distilled water, then dried and weighed. The acids were recovered from the combined washings and soap solution by HCl acidification and ethyl ether extraction. The extract was demineralized by washing with water, and the fatty acids were obtained by removal of ether at reduced pressure and elevated temperature under an atmosphere of nitrogen.

Fatty Acid Methyl Esters. Six g of mixed methyl esters were prepared by reacting the fatty acids with diazomethane (1) for use in gas-liquid chromatography and for physical property measurements.

Fatty Alcohols. Sodium reduction of the triglycerides to fatty alcohols was carried out by following the procedure of Hansley (7). A slight modification was introduced for the hydrolysis of the sodium alkoxide and recovery of the fatty alcohol. The alkoxides (0.63 mole) were hydrolyzed in the original reaction flask by refluxing first with 100 ml of water and then with 1% excess of HCl. The acidulated system was cooled and washed with ethyl ether to extract all the organic compounds. The extract was washed with water and concentrated at ca. 20-mm pressure and ca. 150C pot temperature. The fatty acids present in the concentrate were removed by (a) extraction from ethyl ether with dilute sodium hydroxide (0.5*N*) and (b) adsorption on an activated alumina column with ethyl ether as eluting solvent. The fatty alcohols thus purified were washed with water and concentrated to constant weight at ca 20 mm and 150C.

Wax Esters. Xylene as solvent and *p*-toluenesulfonic acid monohydrate (mp 104–106C) as catalyst were used for the esterification reaction. The weight and mole ratios of fatty acids, fatty alcohols, solvent,

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TABLE I

Composition of Fatty-Acid Methyl Esters and Fatty Alcohols Derived from *Limnanthes douglasii* Seed Oil. Analyses by GLC (11)

Parent acid	Methyl esters			Alcohols		
	Percent by area	ECL		Percent by area	ECL	
		Apiezon L	Resoflex 446		Apiezon L	Resoflex 446
Tetradecanoic	Trace	14.0	14.0	0.1	14.0	14.0
Hexadecanoic	0.2	16.0	16.0	0.3	16.0	16.0
Hexadecenoic	0.2	15.7	16.4	0.3	15.7	16.4
Octadecanoic	Trace	18.0	18.0	0.1	18.0	18.0
Octadecenoic	2	17.7	18.4	1	17.7	18.4
Octadecadienoic	0.2	17.6	19.0	0.4	17.6	19.1
Eicosanoic	2	20.0	20.0	2	20.0	20.0
5-Eicosenoic	65	19.7	20.4	64	19.7	20.4
Eicosadienoic	0.4	19.4	20.6	0.3	19.4	.....
Docosenoic	20	21.7	22.4	21	21.7	22.4
5,13-Docosadienoic	10	21.4	22.6	10	21.4	22.8

and catalyst were: 1.15, 1.00, 34.5, 0.0093; and 1.10, 1.00, 100, 0.015, respectively. The system was refluxed under nitrogen for 9 hr with continuous extraction of water by a cold finger in a modified Bidwell-Sterling distilling receiver. The solution was washed with water to remove the catalyst. The solvent and water were removed by vacuum distillation at ca. 20 mm and 150C. Excess fatty acids were removed as in the preparation of fatty alcohols.

The procedure reported by Ogg et al. (12) was used for hydrogenation of the wax esters. The oils from *Limnanthes douglasii* seeds and jojoba nuts were hydrogenated similarly.

Analytical Methods

Free Fatty Acids in Oil. Half a gram of oil weighed to 0.1 mg was dissolved in 2.00 ml of purified ethanol and titrated with 0.1*N* standard aqueous sodium hydroxide under a stream of nitrogen. Phenolphthalein was used as the indicator. Duplicate blanks and samples were run. Acidity is reported as the acid value.

Iodine Value. AOCS Official Method Cd 1-25 (Wijs method) (10) was followed.

Hydroxyl Content. AOCS Tentative Method Cd 13–60 (10) was used. The values are reported as percentage hydroxyl.

Gas-Liquid Chromatography of Fatty Acid Methyl Esters and Fatty Alcohols. Experimental and identification procedures have been reported earlier (11). Reference standards were the saturated C_{14}–C_{20} and C_{22} methyl esters and saturated C_{14}, C_{16}, and C_{18} primary alcohols.

Density. The volume of the micropicnometer was calibrated with water at 25C. Density is reported as grams per milliliter.

Freezing Point. The freezing point was expressed as the temperature range from first formation of solid particles to development of a thick paste or crystalline mass which resisted flow under force of gravity. The temperature differential between the ethanol bath and sample was maintained at less than 2C. A thermometer was inserted into the sample for direct reading while simultaneously acting as a stirrer.

Viscosity. A Cannon-Fenske-type 0.5-ml. capacity viscometer was made from a 1-mm I.D., 6-mm O.D. glass tubing and calibrated by use of 4 oil samples of known viscosity from the U. S. Bureau of Standards. The calibration curve and the viscometer constants calculated from the standard samples indicated high accuracy of the viscometer over the range of 10 to 80 centipoises ($k = 0.292 \pm 0.001\frac{\text{cp.} \times \text{ml}}{\text{g} \times \text{sec}}$).

Refractive Index. The Bausch and Lomb "Abbe-3L" refractometer (17) was used to determine refractive indices at 25 and 40C.

Results

Limnanthes douglasii Oil. The yield of oil from 1.5 kg of ground seed was 24.7% (26.6% dry basis). The turbidity, which formed upon cooling, disappeared after the oil stood for several days, and a clear orange-brown liquid was obtained with a layer of brownish solid at the bottom of the container. The proportion of this precipitate, estimated by visual inspection, is about 5% at room temperature. No characterization was made of the precipitate, which was reincorporated with the liquid portion for all subsequent work by warming.

Limnanthes douglasii Fatty Acids. The neutral equivalent of the mixed acids was 320 ± 3 (4 samples). Calculations based on the saponification value of 168 showed the yield of recovered acids to be 98% of theory. The amount of unsaponifiable matter was 0.90% of the oil.

Limnanthes douglasii Fatty-Acid Methyl Esters. The composition of the mixed methyl esters as determined by gas-liquid chromatography (GLC) is given in Table I. Equivalent chain lengths (ECL) (11) used for identifying chromatographic peaks are also listed.

Limnanthes douglasii Fatty Alcohols. The total of mixed alcohols recovered after hydrolysis and deacidification of the sodium reduction product was 94% of theory. The acid value of the alcohols was reduced from 4.02 to 0.00 by passing them through an activated alumina adsorption column. This composition as determined by GLC analysis is given in Table I. The ECL of the alcohols were identical to those of methyl esters except for the dienyl alcohols being slightly greater in the Resoflex 446 polyester column.

Limnanthes douglasii Wax Esters. The recovery of the purified liquid wax esters was 91% of the calculated yield. The solid, hydrogenated wax esters were recovered quantitatively; the melting point was 66–68C. The hydrogenation product of the original oil was a hard, glossy, grayish wax melting at 70–72C.

Physical and Chemical Constants of Limnanthes douglasii Oil and Its derivatives. Table II lists some of the constants determined for the oil and its derivatives. The freezing point ranged from +18C for the fatty acids to −18C for the methyl esters. The viscosities of the fatty acids, alcohols, and wax esters were nearly identical, whereas the viscosity for the original oil was much higher and for the methyl esters much lower.

The I.V. determined for the fatty acids is in good agreement with the value 87, calculated according to the composition listed in Table I. The alcohols had a value somewhat higher than 91, also calculated from the composition given in Table I. The I.V. for the

TABLE II

Physical and Chemical Constants of Oil Derivatives from *Limnanthes douglasii* Seeds

Sample	Freezing point, C.	Density, g/ml at 25C	Viscosity, centipoise at 25C	Refractive index η_D^{25}	Refractive index η_D^{40}	Acid value	Iodine value	Hydroxyl, %
Oil	7 to −1	0.905	85	1.4697	1.4646	1.4	87	....
Fatty acids	22 to 18	0.891	38	1.4613	.....	174	89	....
Methyl esters	−5 to −18	0.878	8	1.4542	.....	0.0	....	....
Alcohols	16 to 13	0.857	35	1.4622	.....	0.0	97	5.0
Wax esters	5 to 3	0.866	33	1.4656	1.4602	0.3	96	0.03

wax esters is estimated at 91 if the composition is assumed as identical to the acid and alcohol reactants. Similarly, the percentage hydroxyl calculated for the alcohols is 5.6, higher than the experimental value.

Discussion

Limnanthes douglasii seed oil is unique both in length and in structure of its component fatty acids. It contains fatty acids with *cis*-5 unsaturation (13) and a diunsaturated acid, which has its double bonds separated by six methylene units (2). These acids constitute 83% of the total fatty acids.

The most remarkable feature of this oil, however, is that 97% of the total fatty acids is C_{20} or greater. This percentage is the highest found among the 400 seed oils containing glyceride fatty acids that have been investigated at the Northern Laboratory. The chain length distribution of the fatty acids closely resembles that of hydrolytic fragments from jojoba oil, which is predominantly C_{40} to C_{44} wax esters of C_{20} to C_{22} fatty acids and fatty alcohols. A comparison between the fatty acids and fatty alcohols derived from *Limnanthes douglasii* seed oil in our laboratory and those of jojoba oil as reported by McKinney and Jamieson (9) is shown below:

	Component fatty acids and alcohols from *Limnanthes douglasii*	Component fatty acids and alcohols from Jojoba oil
	%	%
Fatty acids, C_{20} or greater	97	98.1
C_{20} monoene	65	64.4
C_{22} monoene	20	30.2
C_{22} diene	10	.....
Fatty alcohols, C_{20} or greater	97	100.0
C_{20} monoene	64	29.0
C_{22} monoene	21	67.0
C_{22} diene	10	.....
C_{20} monoene	....	4.0

The fatty acid compositions are very nearly identical, except the breakdown of C_{22} into mono- and dienoic acids for *Limnanthes*. The major fatty alcohol in jojoba is erucyl, and the ratio of C_{20} to C_{22} alcohols is inverse to the ratio of the acids. The total percentage of C_{20} plus C_{22} alcohols, however, is again nearly identical to that of *Limnanthes* C_{20} and C_{22} alcohols. The physical properties of the wax esters prepared from the acids and alcohols of *Limanthes* should, therefore, be very similar to those of jojoba oil. A comparison of some physical properties is shown below:

	Limnanthes wax esters	Jojoba oil
Freezing point, C	5 to 3	12 to 7
Density, g/ml, 25C	0.866	0.860
Viscosity, centistokes, 25C	38.6	58.4
37.8C(100F)	25.2	27.0
Refractive index, 25C	1.4656	1.4650
Melting point after hydrogenation, C	66 to 68	66 to 68

The fp and viscosity of jojoba oil are higher, due perhaps to the higher average molecular weight of the alcohol fraction of jojoba oil. At 100F the viscosities of both wax esters are much closer and probably are identical at a slightly higher temperature. Preliminary measurement has shown both to boil over a range of approximately 395–420C at 757 mm. The crystalline wax esters prepared from *Limnanthes* and jojoba by hydrogenation were identical in physical appearance and melting point.

The similarity in properties between jojoba oil and *Limnanthes* wax esters suggests the possibility of the latter supplementing the demand for liquid waxes such as jojoba oil. On a laboratory scale, it was possible to convert 82% of the extracted *Limnanthes* oil to liquid wax esters. The theoretical conversion is 90%.

Limnanthes oil is turbid and viscous at room temperature but is clear and quite fluid at elevated temperatures, e.g., 100–180C. Its potential as a high-temperature lubricant therefore should not be overlooked. The oil is also a good source for production of glutaric and *n*-pentadecanoic acids. Solid triglycerides prepared by directly hydrogenating the oil have a high gloss and an mp comparable to carnauba, 78–85C, and candelilia, 60–72C, waxes (15). The Brinell hardness number was 0.75 at 25C when a 4.0 kg load on a 10.0 mm diameter steel ball was applied for 60 sec. Under identical conditions carnauba wax (mp 76–84C) had a value of 2.6. The simplicity of preparation makes this solid triglyceride wax a good subject for utilization research.

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REFERENCES

1. Arndt, F., in "Organic Synthesis," Coll. Vol. II, John Wiley and Sons, Inc., New York, 1943, p. 165.
2. Bagby, M. O., C. R. Smith, T. K. Miwa, R. L. Lohmar, and I. A. Wolff, J. Org. Chem., *26*, 1261–1265 (1961).
3. Bailey, L. H., "Manual of Cultivated Plants," The Macmillan Co., New York, 1949, *p.* 625.
4. Daugherty, P. M., H. H. Sineath, and T. A. Wastler, Engineering Experiment Station of the Georgia Institute of Technology, Bulletin No. 17, 1953.
5. Earle, F. R., E. H. Melvin, L. H. Mason, C. H. VanEtten, I. A. Wolff, and Q. Jones, JAOCS, *36*, 304–307 (1959).
6. Fore, S. P., F. C. Magne, and W. G. Bickford, JAOCS, *35*, 469–472 (1958).
7. Hansley, V. L., Ind. Eng. Chem., *39*, 55–62 (1947).
8. Knoepfler, N. B., and H. L. E. Vix, J. Agr. Food Chem., *6*, 118–121 (1958).
9. McKinney, R. S., and G. S. Jamieson, Oil & Soap, *13*, 289–292 (1936).
10. Mehlenbacher, V. C., T. H. Hopper, and E. M. Sallee, editors. Official and Tentative Methods, 2nd ed., revised to 1960, American Oil Chem. Soc., Chicago, Ill. (1959).
11. Miwa, T. K., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal. Chem., *32*, 1739–1742 (1960).
12. Ogg, C. L., and F. J. Cooper, Anal. Chem., *21*, 1400–1402 (1949).
13. Smith, C. R., M. O. Bagby, T. K. Miwa, R. L. Lohmar, and I. A. Wolff, J. Org. Chem., *25*, 1770–1774 (1960).
14. VanEtten, C. H., R. W. Miller, I. A. Wolff, and Q. Jones, J. Agr. Food Chem., *9*, 79–82 (1961).
15. Warth, A. H., "Chemistry and Technology of Waxes," 2nd ed., Reinhold Publishing Corp., New York, 1960, pp. *163*, *190*.
16. Wolff, I. A., and Q. Jones, Chemurgic Dig., *19*, No. 7, 4–9 (1960).
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