did not strikingly decrease the time required for cleaning. The action of commercial dishwashing compounds seemed to have a greater temperature gradient than that of an alkaline dishwashing compound.

The addition to a detergent bath of nonyl-phenol-polyglycol ether (Hostapal W) in extremely low concentrations caused a definite increase in the rate of detergent action.

The application of ultrasonic energy to a given detergent bath increased the rate of detergent action as much as 30-fold.

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Search for New Industrial Oils. VIII. The Genus *Limnanthes*

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Abstract

Seed oils from most of the known species and varieties of *Limnanthes* were analyzed for their fatty acid content. Each contained at least 95% acids with more than 18 carbon atoms. The major component acid, *cis*-5-eicosenoic, ranged 52–77% of the acids present. Seeds of all species examined contained thioglucosidic precursors of volatile isothiocyanates, liberated by the action of mustard seed enzymes on the meal. One species also yielded a small amount of an oxazolidinethione-like compound of the type associated with enzyme-treated rapeseed meal.

Introduction

OIL FROM SEED of *Limnanthes douglasii* R.Br. was reported as unusual because 94% of its acids exhibited longer retention times than linolenic acid, the slowest C_{18} component of common oils, in GLC on a polar substrate (4). More detailed investigation led to characterization of the major components as three new acids: *cis*-5-eicosenoic; *cis*-5-docosenoic; and *cis*-5, *cis*-13-docosadienoic (1,14) in addition to the known *cis*-13-docosenoic (erucic) acid. The unusual position of unsaturation in these long-chain acids and the very small quantity of acids containing less than 20 carbon atoms suggest that the oil may be a useful new raw material for processing into industrial products. Exploratory studies on wax esters prepared from *L. douglasii* seed oil indicate their striking similarity to the liquid wax of jojoba seeds (12).

Profitable use of the oil-free meal could have significant bearing on the industrial acceptance of a new oilseed. The value returned by the meal could be reflected in lower prices for the oil, thus improving the competitive position of the oil and making it economically acceptable for a broader variety of applications. Since meal from *L. douglasii* contains lysine and methionine in amounts comparable to the legumes (15), it should have value as a component of feed mixes. However, *m*-methoxybenzyl isothiocyanate, produced by enzymatic hydrolysis of a thioglucoside in *L. douglasii* seed (5), if not removed, may be detrimental to the quality of the seed meal in the same manner that similar compounds affect the use of mustard meals (2). Such removal might effectively be carried out by a process recently developed at the Northern Laboratory that eliminates volatile allyl isothiocyanate from mustard meal (13).

The potential value of *Limnanthes* as an oilseed prompted a study of variation in oil, protein, and thioglucoside contents and in composition of the oil in seed from all available species.

Botanical Nature of the Genus

The genus *Limnanthes* as classified by Mason (9) includes eight species of annual herbs native to the Pacific Coast. All species germinate in the fall or winter and require relatively cool weather during the growing season. Hot weather in late spring adversely affects them. An ecological survey of the wild populations disclosed a wide range among varieties in adaptability to different soil and moisture conditions. Such diversity indicates promise for successful introduction to cultivation. Also there is sufficient variability in growth form and seeding characteristics to indicate success for the selection of superior strains for commercial production. Genetic crosses between several varieties have already been demonstrated (9). This compatibility is important in developing cultivated varieties; nearly all cultivated crops result from crossing varieties and species either intentionally or inadvertently. *L. douglasii*, a garden ornamental, is the only cultivated species. Figure 1 shows a closeup of a cultivated plant and a dense wild stand of *L. douglasii* var. *nivea*. The botanical aspects of this genus as a potential source of new oilseed crop will be published elsewhere (6).

Materials and Methods

Seeds used in the present survey, except for a few samples of *L. douglasii* bought from a commercial supplier, were obtained by collection from wild plants in the spring of 1962.

Seeds were cleaned and analyzed as previously reported (4). Methanolysis of the oil with HCl as catalyst yielded methyl esters of the fatty acids, which were analyzed by GLC (10). Results are reported as percentage of the area under the curve attributable to a given methyl ester.

Enzyme treatment of the seed meals and estimation of the liberated volatile isothiocyanates and oxazolidinethiones were performed essentially as described by Wetter (16,17). A slight modification (3) allowed investigation by paper chromatography of a portion of the thiourea derivatives of the steam-distilled isothiocyanates (8).

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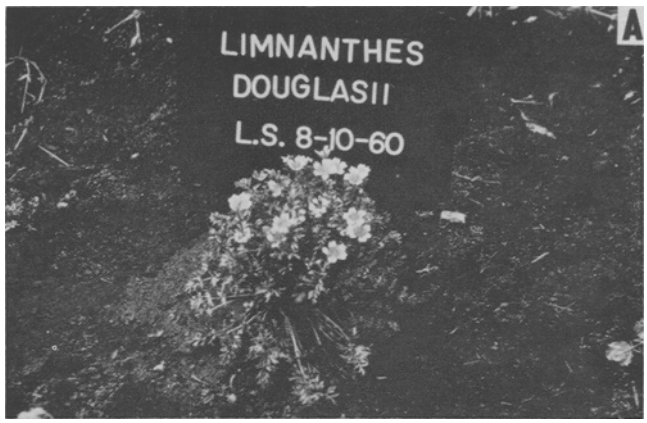

FIG. 1A. A cultivated plant of *Limnanthes douglasii*.

FIG. 1B. A stand of *Limnanthes douglasii* var. *nivea*.

Results and Discussion

Oils from all samples analyzed (Table I) are similar in their overall composition to oil in the original sample from *L. douglasii;* i.e., less than 5% of the component acids are the common C_{16} and C_{18} acids. The major component in all samples ranges 52–77% and, on the basis of equivalent chain lengths (ECL) (11) from GLC, is undoubtedly the same as the *cis*-5-eicosenoic acid discovered in *L. douglasii* (14). The other major components, similarly identified by their ECL, are the C_{22} monoenes (8–29%) and *cis*-5, *cis*-13-docosadienoic acid (7–20%). Presumably both the *cis*-5- and *cis*-13-docosenoic acids are present in all species as in the sample first investigated (14), but GLC on the packed columns used failed to separate them. In addition to the 5,13-C_{22} dienoic acid, which (as the methyl ester) has ECL of 21.4 (Apiezon L) and 22.6 (Resoflex R-446), oil from several samples contained small amounts of a component with ECL of 21.7 (ApL) and 23.0 (R-446). Since these ECL differ from those of methyl behenate by an amount equal to the differences between linoleate and stearate, the component is probably a methylene-interrupted C_{22} dienoic acid. The small amount of this component in the oil does not justify isolation and characterization at this time.

In the 14 meal samples examined the quantity of volatile isothiocyanates produced ranged 11.0–19.3 mg of isothiocyanate per g solvent-extracted meal, calculated as methoxybenzyl isothiocyanate. The thiourea derivatives of these isothiocyanates were chromatographed on paper. Each sample produced only one thiourea. In 10 samples, the R_{ph} (migration relative to phenylthiourea) was 1.0; and in two (*L. bakeri* and one sample of *L. douglasii* var. *nivea*), it was 0.8. As the thiourea of *m*-methoxybenzyl isothiocyanate, produced from *L. douglasii,* migrates with an R_{ph} of 1.0 (5), probably the volatile isothiocyanate in 10 of the present samples is the *m*-methoxybenzyl compound. The known presence in a different plant family, the Cruciferae, of both *p*-methoxybenzyl- and 4-methylthiobutyl isothiocyanates (7), the thioureas from which also migrate with an R_{ph} near 1.0, suggests a need for definite confirmation of this tentative identification by isolation and comparison with authentic specimens or their appropriate properties.

No further data were obtained on the unknown thiourea with R_{ph} of 0.8. Its occurrence in only one of the samples of *L. douglasii* var. *nivea* is particularly unexpected. This exception may result from unusual environmental conditions during growth, or the sample might even be in a different botanical category than originally assigned, yet so similar that it has escaped separate classification.

Of the 14 samples analyzed for oxazolidinethiones, only one (*L. alba*) contained any. It had 2.7 mg per g extracted meal when calculated as vinyl oxazolidinethione.

TABLE I

Analytical Data on *Limnanthes* Seed and Oil

Species and variety	Seed analysis			Oil properties		Composition of methyl esters, % (area percentage by GLC)															Derivatives from meat thioglucosides		
																					Volatile isothiocyanate		
	Wt/ 1,000 g	Protein NX6.25 % DB	Oil % DB	Iodine Value (Wijs)	Refractive Index n_D^{40}	$C_{22:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:1}$	$C_{22:2}$[a]	$\Delta 5,13$ $C_{22:2}$	$C_{22:3}$	Others[b]	Amount mg/g[c]	Chromatographic mobility as thiourea derivative R_{ph}[d]	Oxazolidinethione mg/g[c]
L. alba Benth.	5.1	20	27	94	1.4653	0.2	0.1	0.2	0.2	0.1	1	0.3	0.3	0.7	61	15	tr	20	0.1	0.6	15.6	1.01	2.7
L. alba var. *versicolor* (Greene) C. T. Mason	7.1	19	31	87	1.4644	tr	0.1	0.1	0.2	0	1	0.1	0.2	0	60	28	0	10	0.2	0.3	19.3	1.00	0
L. bakeri J. T. Howell	4.1	16	26	88	1.4646	0.1	0.2	0.3	0.3	0.1	3	0.4	0.4	1	57	25	0.7	11	0.7	0	14.2	0.83	0
L. douglasii R. Br. (refs. 1,14)	7.6	25	25	87	1.4628	tr	tr	0.4	0.3	0.3	2	0.7	0	1	64	20	0	10	0	1	.....	.....	..
L. douglasii R. Br.	7.6	16	30	87	1.4645	0.1	0.1	0.3	0.2	0.2	1	0.4	0.3	1	65	20	0	11	0	0	18.0	1.02	0
L. douglasii R. Br.	6.6	17	25	87	1.4645	0	0	0.2	0.2	tr	3	1	0.4	2	58	19	0.5	15	0.1	0.4	.....	.....	..
L. douglasii R. Br.	5.7	20	28	87	1.4647	0.1	0.1	0.3	0.3	0.1	1	0.3	0.2	1	62	18	0.4	15	0	0.9	.....	.....	..
L. douglasii var. *nivea* C. T. Mason	5.0	17	26	86	1.4644	0	0	0.2	0.2	0.3	2	1	0.5	2	62	22	0	7	2	1	14.0	0.82	0
L. douglasii var. *nivea* C. T. Mason	4.3	19	30	86	1.4646	tr	0.1	0.4	0.3	0.1	2	0.3	0.3	2	65	18	0.5	10	0	2	.....	.....	..
L. douglasii var. *nivea* C. T. Mason	7.6	22	30	87	1.4645	0.1	0.1	0.2	0.2	0.1	0.8	0.2	0.2	0.9	67	23	0	7	0	0	16.4	1.00	0
L. douglasii var. *nivea* C. T. Mason	6.5	15	33	85	1.4642	0.1	0.1	0.2	0.2	0.3	1	0.5	0.2	1	60	24	0.5	11	0	1	12.6	1.01	0
L. douglasii var. *rosea* (Benth.) C. T. Mason	4.3	16	20	86	1.4639	0.1	0.2	0.3	0.3	0.3	1	0.6	0.6	1	72	12	0.5	10	0.8	0	12.3	1.00	0
L. douglasii var. *rosea* (Benth.) C. T. Mason	5.5	17	22	91	1.4652	0.1	0.2	0.4	0.2	0.1	1	0.2	0	0.5	77	8	0	12	0	0.8	11.0	0.99	0
L. floccosa Howell	6.5	25	28	89	1.4645	0.1	0.1	0.2	0.2	0	0.9	0.6	0.4	1	59	24	0	12	0.9	1	15.0	0.99	0
L. gracilis Howell	5.5	17	29	89	1.4648	tr	tr	0.3	0.4	0.2	1	0.2	0.1	1	55	29	0	13	0.1	0	13.1	0.99	0
L. gracilis var. *parishii* (Jepson) C. T. Mason	4.5	18	33	89	1.4647	tr	tr	0.2	0.3	0.1	2	0.1	0.4	1	59	24	0	12	0	0	14.9	.....	0
L. montana Jepson	3.2	21	26	89	1.4648	tr	tr	0.3	0.3	0	0.8	0.4	0.3	1	52	25	0.6	17	0.8	2	15.0	1.01	0
L. striata Jepson	4.0	20	29	90	1.4646	0.2	0.2	0.8	0.3	0	2	0.6	0.5	0.3	65	14	0.3	16	0.2	0.4	18.2	.....	0

[a] See text.
[b] This figure includes any $C_{10:0}$, $C_{20:2}$, $C_{20:3}$, C_{24} and unknowns which might be present.
[c] Mg/g extracted meal, calculated as methoxybenzyl isothiocyanate.
[d] Migration relative to phenyl thiourea. Component at $R_{ph} = 1.00 \pm 0.03$ is probably *m*-methoxybenzyl thiourea; that at $R_{ph} = 0.8$ is unidentified.
[e] Mg/g extracted meal, calculated as vinyl oxazolidinethione.

All the seed samples were relatively rich in oil (20–33%). The calculated protein content of the oil-free meal on a dry basis, 21–34%, is lower than that of the usual oilseed meals, but adequate to be useful as a feed material. Should profitable uses develop for this oil and meal, the variability of germ plasm in this collection offers hope that a breeding program could develop plant forms suitable for mechanical production and harvesting and possibly also could vary the oil composition to increase its value for specific applications.

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The Determination of Hydrophile-Lipophile Balance by Gas-Liquid Chromatography[1]

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Abstract

Measurement of the relative retention time of a pair of liquids, one polar and one nonpolar, permits determination of the polarity of the liquid chromatographic substrate. It is shown that this polarity is a measure of the hydrophile-lipophile balance (HLB) when the substrate is a surface-active agent. The effects of structure, additivity in mixtures, and free polyol are discussed. A GLC apparatus, designed to permit the rapid determination of HLB by this technique, is described.

Introduction

EVER SINCE the concept of HLB-number was introduced by Griffin (1) as a measure of the polar character of emulsifier molecules, attempts have been made to relate this quantity to some basically surface-chemical property of the emulsifying agent. This has been done with two ends in view: first, to attempt to place this admittedly pragmatic index on a firmer theoretical basis and, secondly, to find a method for the rapid and easy determination of the HLB number. Earlier work in this area has been summarized by one of us (2). More recently, attempts have been made to correlate HLB-number (or equivalent indices) with liquid-liquid distribution data (3), stability to centrifugation (4), and to interfacial tension (5).

Employing a simplified picture of the mechanism of emulsion breakdown, Ross, Chen, Becher, and Ranauto (6) demonstrated that a correlation existed between spreading coefficient and HLB-number. It was possible to show that for a given two-phase system the HLB for maximum emulsion stability corresponds to a spreading coefficient which is slightly negative (for oil-in-water emulsions, at least).

Davies (7) has demonstrated that coalescence rates, as measured by the technique of Cockbain and McRoberts (8), can be correlated to HLB-numbers, and that a thermodynamic extension of these observations permits us to calculate HLB-numbers as a sum of structural factors, much in the same way as done with the parachor.

Davies (9) has also shown recently that the HLB-number may be related to the phase ratio at which inversion of an emulsion occurs under dynamic conditions, and has designed an emulsator which permits ready determination of this parameter.

However, recent progress in the area of analytical chemistry, notably in the development of the techniques of GLC, suggests that a more-or-less direct measurement of the polarity (hence, implicitly HLB-number) of the surface-active molecule may be possible, without having recourse to a specifically surface-chemical property. As is well known, the ability of a gas-chromatographic "substrate" to separate the components of a sample depends on the polarity of the substrate with respect to the components of the sample. From this point of view, the use of a surface-active agent as a substrate in GLC might well enable one to carry out a direct determination of HLB-number.

In 1959, Harva, Kivalo, and Keltakallio (10) reported that if one employed a nonionic surface-active agent as the liquid substrate in GLC, there was a linear relationship between the partition coefficient for a particular chromatographic sample material and HLB-numbers of the various substrates. The sample materials studied were water and diisobutylene.

For water, they found that two approximately parallel lines of positive slope were obtained for sorbitan fatty acid esters and polyoxyethylated sorbitan fatty acid esters, respectively, while with diisobutylene a single straight line of negative slope was obtained.

In the last year, Huebner (11) has suggested that a more meaningful measure of polarity is the "polarity index." This is based on the determination of an "apparent carbon number" for methanol. It depends on the well-known fact that the logarithm of the retention volume of each member of a homologous series is a linear function of the number of carbon

[1] Presented at National Colloid Symposium, Ottawa, June, 1963.