lactic acid. The esterification of 3 moles reagent grade lactic acid with 2 moles glycerol yielded a mixture of glycerol lactates that were added to shortening on the basis of their saponification equivalent. Reagent grade lactic acid was also added to shortening in relation to the lot analysis. The results show in Table I.

Precision. The estimation of precision given in Table II was obtained from ten WICLA determinations on a commercial lactylated shortening.

Determination of Similar Acids. As new fat emulsifiers which contain low mol wt hydroxylated acids appear and require analysis, the present method might be adapted for this purpose. For instance, in a preliminary experiment, 0.2% citric acid in shortening has been eluted qualitatively from this system by a mixture of 55 volumes *n*-butanol and 45 volumes chloroform.

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Search for New Industrial Oils. XI. Oils of Boraginaceae

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Abstract

Analysis of seed oils from 29 species of the family Boraginaceae revealed widespread occurrence of 6,9,12-octadecatrienoic and C_{18} nonconjugated tetraenoic acids in addition to linolenic and other common C_{16} and C_{18} acids. The 6,9,12-octadecatrienoic acid ranged in amount from 0–27%, tetraene from 0–17%, and linolenic acid from 0.3–50%. Iodine values of the oils ranged from 88–225.

Introduction

ANALYSIS of seed oils from an extensive sampling of the plant kingdom reveals that the family Boraginaceae is unique in the frequency with which nonconjugated tetraenoic acids occur. In the first paper (2) from this continuing program two species in the family were reported to contain small amounts of tetraene on the basis of ultraviolet absorption after isomerization by alkali. In later samples, oil was analyzed by gas-liquid chromatography (GLC), and the tetraene was identified as an 18-carbon acid. Furthermore, the trienoic fraction was shown to include two components. One had the equivalent chain length (ECL) of the usual linolenic acid and the other the ECL of the 6,9,12 isomer (4), long known only in oil from the genus *Oenothera,* family Onagraceae but recently reported from *Humulus lupulus,* family Moraceae (5). The tetraenoic acid has been identified as 6,9,12,15-octadecatetraenoic acid in oils from *Echium plantagineum* (6) and *Onosmodium occidentale* (1), and our GLC identification of the 6,9,12-octadecatrienoic acid has been confirmed.

This paper contains the analyses of seeds and oils from 29 species in the borage family.

Materials and Methods

The plant family Boraginaceae includes some 100 genera and 2000 species, mostly herbaceous and often perennial. There are five subfamilies, one of which is divided into five tribes (7). Four of the subfamilies and all five tribes are represented in this paper. Numerous species are grown as ornamentals, but we do not know of any studies directed toward large-scale seed production for industrial use. Seed samples were provided by the U. S. Dept. of Agriculture's Crops Res. Div. as obtained by staff botanists from wild plants, by botanists under contract in various parts of the world, or by purchase from commerical seed suppliers.

Seeds were cleaned and analyzed as previously described (2). The components analyzed included seed plus pericarp in all instances except two, *Paracaryum angustifolium* and *Lappula redowskii,* for which seed only was analyzed. Methyl esters were prepared from the oils by HCl-catalyzed methanolysis.

Each ester preparation was analyzed by GLC at least three times in equipment described previously (3). A rapid exploratory analysis was made in a 125 x 0.3 cm column to identify the slowest moving components. Then each ester preparation was analyzed on two different columns: a 200 x 0.6 cm glass column containing 20% Apiezon L on 60–80 mesh Celite 545 at 258C with a helium flow of 90 ml/min and a 200 x 0.6 cm glass column containing 20% LAC-2-R 446 on the same support at 196C with flow rate of 120 ml/min. In the polar column the peak of the C_{18} tetraene (ECL 20.1) overlapped the C_{20} esters. However, in the nonpolar column as operated, the C_{18} tetraene (ECL 17.4) was widely separated from the C_{20} esters but coincided with the 6,9,12-triene (4) and was not fully separated from the usual C_{18} unsaturates. The percentage of tetraene was determined by subtracting the C_{20} methyl esters (ECL 19.7–20.0) from the Apiezon L chromatogram from the methyl esters in the C_{20} region from the LAC-2-R 446 column (ECL 20.0–20.4). The percentage of 6,9,12-triene (ECL 19.3 LAC-2-R 446 column) was determined from the LAC-2-R 446 chromatogram where separation was complete. When the nonpolar column was operated at 210C with a flow rate of 116 ml/min, the peak representing the tetraene and 6,9,12-triene was separated from that of the usual C_{18} unsaturates and the amount agreed with the sum of these two components determined by the procedure described.

Results and Discussion

In all but three of the samples analyzed (Table I), GLC shows the presence of C_{18} tetraene, C_{18} 6,9,12-triene, or both. The exceptions are *Lithospermum officinale, Ehretia acuminata,* and *E. aspera,* whose

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TABLE I

Analytical Data on Boraginaceae Seeds and Oils

Source	Wt/1,000, g	Seed Analysis: Oil content % D.B.	Seed Analysis: Protein content Nx6.25, % D.B.	Oil Properties: Iodine value	Oil Properties: Refractive Index n_D^{65}	Composition of Methyl Esters, %: 16:0	18:0	18:1	18:2	18:3	18:3[6,9,12]	18:4	20:1	22:1	Other components
Cordioideae															
Cordia obliqua Willd.	594	4	8	88	1.4640	18	7	45	27	0.5	0.1	0	0.1	0.3	2
Ehretioideae															
Ehretia acuminata R. Br.	13	5	10	140	1.4758	8	5	18	67	0.7	0	0	0.4	0	2
Ehretia aspera Roxb.	28	5	10	119	1.4690	11	8	23	55	2	0	0	0.8	0	1
Heliotropioideae															
Heliotropium europaeum L.	0.8	24	21	131	1.4656	7	3	20	69	0.3	0	0.2	0	0	0.2
Borsginoideae															
Cynoglosseae															
Cynoglossum amabile Stapf. & Drumm.	5.5	23	18	123	1.4683	10	2	28	25	4	11	0.7	5	7	8
Cynoglossum officinale L.	25	21	20	128	1.4682	6	1	34	26	7	6	3	5	8	5
Cynoglossum pictum Soland.	25	22	17	114	1.4668	6	1	47	12	9	3	0.2	6	8	7
Pentaglottis angustifolium Boiss.	5.0	31	24	133	1.4684	6	2	35	23	12	6	3	5	6	2
Pentaglottis caelestinum Benth. & Hook.	5.0	21	19	125	1.4684	11	1	28	28	3	12	1	4	8	3
Eritrichieae															
Cryptantha bradburiana Payson	1.9	27	21	166	1.4719	8	2	33	13	21	8	10	3	2	0.8
Cryptantha angustifolia (Torr.) Greene	0.3	33	26	184	1.4738	9	3	17	23	36	5	6	1	0	0.3
Lappula redowskii (Hornem.) Greene	1.1	19	18	204	1.4764	7	3	18	16	32	5	17	1	0	0.5
Anchuseae															
Anchusa capensis Thunb.	2.2	29	19	157	1.4693	9	2	24	31	17	10	3	2	2	0.3
Anchusa hybrida Ten.	6.0	20	20	149	1.4700	10	2	28	24	13	13	3	4	4	0.2
Borago officinalis L.	21	38	21	139	1.4680	12	4	18	37	0.9	20	0.9	4	2	0.9
Symphytum officinale L.	6.2	21	20	162	1.4716	8	2	15	43	1	27	0.5	2	1	0.3
Lithospermeae															
Cerinthe major L.	50	25	13	127	1.4668	10	5	41	14	24	0.4	0.3	2	0	2
Cerinthe minor L.	11	10	14	194	1.4756	7	2	14	21	56	10	8	0.9	0	0.1
Lithospermum apulum Vahl.	5.6	18	12	211	1.4772	6	2	12	17	41	6	14	1	0	0.1
Lithospermum officinale L.	1.3	26	21	139	1.4665	6	3	17	73	0.4	0	0	0	0	0.8
Lithospermum tenuiflorum L.f.	4.7	16	20	225	1.4787	6	2	19	13	50	4	16	0.9	0	0.2
Moltkia aurea Boiss.	16	10	10	193	1.4743	6	3	16	19	36	10	16	0.5	0	3
Moltkia coerulea Lehm.	22	10	9	193	1.4750	6	3	20	18	35	11	6	0	0	0.6
Myosotis sylvatica Hoffm.	0.6	43	17	155	1.4700	8	2	27	26	13	5	12	5	3	0.2
Onosma sericeum Willd.	17	20	19	171	1.4722	8	3	22	31	18	13	5	0.5	0	0.1
Onosma stellulatum Waldst. & Kit.	3.5	23	24	195	1.4748	8	2	14	21	41	4	9	0.4	0	0.2
Onosmodium molle Michx.	.31	17	12	185	1.4737	8	3	19	19	24	20	6	1	0	0.1
Echieae															
Echium italicum L.	6.3	17	16	204	1.4759	8	3	17	11	39	8	12	0.8	0	1
Echium plantagineum L.	4.4	30	19	197	1.4756	7	4	17	15	34	10	13	0.4	0	0.1

oils lack both of these components and contain very little linolenic acid. *Cordia obliqua* oil contains 0.1% of the 6,9,12-triene but no tetraene, and *Heliotropium europaeum* oil contains 0.2% tetraene but no 6,9,12-triene. In all other samples the tetraene ranged from 0.2–17%, and the 6,9,12-triene from 0.4–27%. All samples contained linolenic acid, with amount ranging from 0.3–50%, as well as palmitic, stearic, oleic, and linoleic acids.

Variability within the family is reflected in iodine values (I.V.) ranging from 88–225. Oil from *Cordia obliqua,* I.V. 88, contains 70% saturated plus monoenoic acids, whereas *Lithospermum tenuiflorum,* I.V. 225, contains 70% C_{18} trienoic plus tetraenoic acids. Variability within the genus *Lithospermum,* while not as great as that within the entire family, is perhaps more striking. Whereas the one species mentioned produces oil containing 70% trienoic plus tetraenoic acids, another has oil with 73% linoleic acid and essentially none of the more highly unsaturated acids. Similar variation is exhibited in the genus *Cerinthe. C. major,* I.V. 127, is almost devoid of tetraene and 6,9,12-triene, but *C. minor,* I.V. 194, contains significant amounts of both.

In view of the variation in oil composition within the tribe Lithospermeae, subfamily Boraginoideae, generalizations regarding composition of the oils of the several groups must be considered as preliminary. In the present sampling of the family, oils from members of tribes Cynoglosseae and Anchuseae contain more 6,9,12-triene than tetraene; those from tribe Eritrichieae have more tetraene than 6,9,12-triene; and those from tribe Lithospermeae are variable. Tribes Cynoglosseae and Anchuseae consistently produce some C_{22} monoenoic acid, while the other tribes contain many members devoid of this component. Samples from the subfamilies Cordioideae, Ehretioideae, and Heliotropioideae contain insignificant amounts of acids more unsaturated than linoleic. In this respect they differ from the subfamily Boraginoideae, which has only one oil of this type, that from *Lithospermum officinale.*

Under "Other Components" in Table I are reported minor constituents of several types. Included are $C_{14:0}$ in 22 samples in amounts up to 0.4%, $C_{16:1}$ in 28 samples up to 0.6%, $C_{20:0}$ in 16 samples up to 1.3%, $C_{22:0}$ in 10 samples up to 1.2%, and unidentified peaks in 12 samples up to 1.5%. Also included are 3–5% $C_{24:0}$ and $C_{24:1}$ in the three samples of *Cynoglossum* and 1.5% of the same acids in the sample of *Moltkia aurea.*

In addition to data in Table I, results of several other tests used routinely in this survey are summarized here. Qualitative tests for starch in the seed and carbonyl components in the oil were negative for all samples. IR and UV absorption gave no evidence of significant amounts of unusual components. Titration with HBr as for oxirane oxygen indicated small amounts of reactive materials, but the largest amount, in *Heliotropium,* was only 2.7% calculated as C_{18} acid, and only four contained as much as 1%. The identity of the reactive material was not investigated.

Oils with high I.V. should serve as drying oils or as raw materials for other applications in which a large amount of unsaturation is important. The 6,9,12-trienoic and the tetraenoic acids may contribute properties to films and reaction products superior to those from the usual high-linolenic drying oils. Indications of industrial interest in these oils could catalyze a program for selection and development of strains suitable for economical production.

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