tane-insoluble solid was crystallized twice from 82% of acetone. The resulting tetrabromostearic acid had an activity of 0.074 d.p.s. per mg. (expressed on the basis of the bromine-free compound). Too little activity remained for further purification.

Discussion

The fish employed in these studies were maintained on Purina Trout Chow until the start of the experiment when they were fed the same diet after ether extraction. Under these circumstances the fish deposited fat which apparently contained high proportions of the typical mammalian polyunsaturated acids, linoleic and arachidonic acids. Linolenic acid, the typical marine fatty acid, is present in very small amounts. It therefore appears in agreement with previous studies (7,8) that the fish deposits, to a significant extent, the fatty acids of the diet. In this case these were the typical animal and vegetable fatty acids furnished by the 4% of fat in the diet.

The longer-chain, more highly unsaturated acids, for which arachidonic may be taken as an example, appear to be formed by desaturation and elongation processes typical of the mammalian metabolism (3). If this is indeed the case, structure determination will probably show them to be derived largely from oleic or linoleic acid and not from linolenic acid as is the case with fish on a natural marine diet (4,5). Thus the same processes of alteration of unsaturated fatty acids found in mammals probably holds for fish with the exception that in the latter case the process continues to produce fatty acids of somewhat greater chain-length and higher degree of unsaturation.

The tracer studies revealed that under conditions of the experiment the fish synthesized large amounts of fatty acids. In agreement with animal studies the rapidly synthesized fatty acids were largely of the saturated type; the polyunsaturated acids were by far the least active.

Degradation studies indicated that the 20-carbon polyunsaturated acid, probably largely arachidonic, was synthesized by addition of acetate to a relatively inactive 18-carbon portion, probably largely derived from linoleic. The nature of the active 18-carbon acid that contributed activity to the linoleic and the terminal 18 carbons of the arachidonic acid was indicated by two experiments. Degradation of the linoleic fraction showed it to contain equal activity in the carboxy and methyl moieties. That the active acid in the fraction was not linoleic is revealed by the fact that the activity did not appear in the recrystallized tetrabromostearic acid derived from the linoleic acid. A suggestion as to its nature can be made. Since it had uniform activity throughout the molecule, it could have been derived from oleic acid (similar degradation of the crude oleic acid fraction from these fish revealed that, unlike mammals, fish appear to synthesize uniformly labelled oleic acid). Furthermore an octadecadienoic acid derived from oleic must be postulated for rats which have been fed a fat-free diet (14). This is the 6,9-octadecadienoic acid, which is a probable intermediate between oleic and 5,8,11-eicosatrienoic acids (14). Another probability is the postulated 8,11-octadecadienoic acid intermediate between palmitoleic and 7,10,13-eicosatrienoic acids (14). The existence of this acid in rats has been demonstrated by Fulco and Mead (3). Either of these acids would have the properties of the active octadecadienoic acid postulated in the present study.

In any event the tracer studies tend to confirm the hypothesis that polyunsaturated fatty acid metabolism is not qualitatively different in fish and mammals. Although small quantities of polyunsaturated acids can be synthesized from acetate, these are apparently not the higher essential fatty acids, which must be formed from dietary linoleic acid.

Summary

Following injection of *Tilapia mossambica* with acetate-1-C^{14}, their fatty acids were isolated, fractionated, and degraded. The high content of linoleic and arachidonic acids was evidently derived from the diet. Degradation of these acids revealed a distribution of carbon-14 similar to that found in similar studies on mammals.

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Search for New Industrial Oils. IV.

F. R. EARLE, C. A. GLASS, GLENDA C. GEISINGER, and I. A. WOLFF, Northern Regional Research Laboratory,[1] Peoria, Illinois; and QUENTIN JONES, Crops Research Division,[2] Beltsville, Maryland

As related in previous papers of this series (1), a program is in progress to determine by chemical screening analyses what amounts and general classes of fatty acids are contained in seed oils of a large number and variety of presently uncultivated species. Those with suitably high oil content, and with fatty acid composition thought to be sufficiently different from that of present commercial vegetable oils to make them of potential practical interest, are then scheduled for more intensive chemical study.

In this paper we report results obtained on 158 species representing 52 plant families in 23 orders. Of these, 138 are previously unreported in the compilations of Hilditch (2) and Eckey (3) or in more

[1] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Agricultural Research Service, U. S. Department of Agriculture.

TABLE I

Analytical Data on Seeds and Derived Oils

Source	Common name	Component analyzed: Plant part (see key)	Component analyzed: Weight of 1,000 g.	Oil content, % D.B.	Protein content (N×6.25), % D.B.	Fatty acid content of oil: Iodine value, Wijs	Saponification value	Refractive index $n^{40/D}$	Triene as C_{18} acid: Nonconj., %	Triene as C_{18} acid: Conj., %	Diene as C_{18} acid: Nonconj., %	Diene as C_{18} acid: Conj., %	Monoene, as oleic, %	Saturates, %	HBr uptake, as C_{18} epoxy acid, %	C=O as C_{18} acid, %	OH as C_{18} acid, %	Infrared
Coniferales																		
Podocarpaceae																		
1. *Podocarpus nagi*	Japanese podocarpus	A	882.0	40	10	157	174	1.4707	0	0	(61)[1]	0	(50)[1]	(-16)[1]	0.4	-[2]	...	Trace-*trans*
Taxodiaceae																		
2. *Cryptomeria japonica*	Japanese cryptomeria	A	4.5	11	11	189	185	1.4769	(41)[1,3]	0	(21)[1]	0	(34)[1]	(-3)[1]	1.9	3.0	...	Usual
Liliales																		
Liliaceae																		
3. *Allium porrum*	Leek	A	3.4	16	26	135	188	1.4690	0	0	61	1.7	24	9	2.1	-	4	Usual
4. *Asparagus officinalis*	Garden asparagus	A	22.1	15	16	132	182	1.4677	0	0	52	0	40	3	0.4	-	...	Usual
5. *Dasylirion wheeleri*	Wheeler sotol	A	1.6	22	26	146	182	1.4678	0	0	73	0	14	8	0.5	0.1	3	Usual
6. *Hemerocallis* (hort. sp.)	Day-lily	B	27.9	27	39	134	181	1.4678	1	0	59	0	26	9	2.7	-	...	Usual
7. *Yucca constricta*	Buckley yucca	A	20.0	26	21	137	176	1.4681	0	0	63	0	24	8	0.3	-	...	Trace ROH
8. *Yucca glauca*	Small soapweed	A	24.5	27	21	142	184	1.4684	0	0	69	0	19	8	0.2	-	...	Usual
Amaryllidaceae																		
9. *Agave schottii*	.....	A	2.2	21	29	142	180	1.4690	0	0	72	0	11	12	0.3	-	...	Usual
Iridaceae																		
10. *Iris germanica*	German iris	A	59.9	17	15	138	129	1.4940	0	0	(21)[1]	0	(111)[1]	(-36)[1]	0.3	5.0	...	High ROH; bands at 6.0, 6.2, and 11.9 μ
Casuarinales																		
Casuarinaceae																		
11. *Casuarina torulosa*	Forest beefwood	A	2.8	42	49	142	180	1.4683	0	0	(56)[1]	0	(44)[1]	(-5)[1]	0.4	-	...	Usual
Urticales																		
Ulmaceae																		
12. *Zelkova serrata*	Japanese zelkova	C	14.0	21	18	13	284	1.4473	0	0	3	0	7	85	0.2	-	...	10% *Trans*
Moraceae																		
13. *Maclura pomifera*	Osage orange	A	21.6	44	38	145	189	1.4689	0	0	72	0	14	9	0.2	-	1	Usual
Santalales																		
Olacaceae																		
14. *Ximenia americana*	.....	A	834.0	62	20	85	150	1.4718	(1/	1/	1/	1/	1/	1/)	0.7	1.0	...	Triple bond; 5% conj. triene
Chenopodiales																		
Chenopodiaceae																		
15. *Kochia scoparia*	Summer-cypress	A	0.6	16	28	135	186	1.4693	5	0.1	48	0	38	5	0.6	-	...	Usual
16. *Salsola pestifer*	Russian thistle	C	1.7	22	40	132	188	1.4686	5	0.2	50	0	30	10	0.7	-	0	Usual
Ranales																		
Ranunculaceae																		
17. *Paeonia brownii*	Browns peony	A	135.0	19	14	155	180	1.4690	34	0	19	0	31	12	0.4	-	2	Usual
18. *Thalictrum revolutum*	Meadow-rue	C	1.5	38	28	175	181	1.4721	1	0	(61)[1]	0	(68)[1]	(-35)[1]	0	-	...	*Trans*
Menispermaceae																		
19. *Menispermum canadense*	Moonseed	D	104.0	16	13	178	186	1.4678	25	0	(46)[1]	0	(29)[1]	(-4)[1]	1.0	-	...	High free acid
Dilleniaceae																		
20. *Actinidia arguta*	Tara-vine	A	1.6	22	16	183	194	1.4763	44[4]	0.3	7	2.1	35	3	4.1	6.0	...	10% ROH; Trace-*Trans*
Calycanthaceae																		
21. *Calycanthus floridus*	Carolina allspice	A	59.4	48	24	134	178	1.4671	0	0	62	0	24	10	0.2	-	...	Usual
22. *Chimonanthus praecox*	Wintersweet	A	109.6	37	34	117	176	1.4657	1	0	52	0	23	20	1.0	-	...	Usual
Lauraceae																		
23. *Sassafras albidum*	Sassafras	D	58.0	60	24	6	276	1.4470	0	0	0	0	6	89	0.6	-	...	Trace-*trans*
Rhoeadales																		
Papaveraceae																		
24. *Argemone hispida*	Crested prickly poppy	A	2.5	32	17	125	171	1.4659	0	0	57	0	24	15	0.5	-	...	Usual
25. *Argemone intermedia*[5]	Prickly poppy	A	2.8	37	18	136	181	1.4672	0	0	65	0	18	12	0.4	-	1	Usual
Capparidaceae																		
26. *Cleome serrulata*	Bee-spider flower	A	2.6	34	21	166	197	1.4711	29	0	38	0	21	8	0.2	-	2	Usual
27. *Cleome spinosa*[5]	Spiny spider flower	A	2.2	36	19	116	180	1.4658	4	0	40	0	36	16	0	-	...	Usual
28. *Isomeris arborea*	Tree burrofat	B	40.8	45	37	107	189	1.4636	1	0	35	0	44	15	0.2	-	...	Usual
29. *Polanisia trachysperma*	Roughseed clammyweed	A	3.0	25	18	140	197	1.4683	2	0	65	0	19	10	0.2	-	...	Usual
30. *Polanisia viscosa*	Clammyweed	A	1.4	26	16	130	189	1.4686	0	0.5	55	0	32	8	0.8	-	29	Usual
Rosales																		
Hamamelidaceae																		
31. *Liquidambar styraciflua*	Sweet gum	A	4.0	30	33	141	182	1.4678	1	0	67	0	20	8	0.6	-	2	Usual
Rosaceae																		
32. *Crataegus mollis*	Downy hawthorn	A	9.0	31	47	128	183	1.4676	0	0.1	50	1.0	38	6	2.3	-	...	Usual
33. *Geum chiloense*	Double crimson avens	A	1.4	24	30	162	186	1.4720	30	0	26	0	37	3	0.3	-	...	Usual

(Continued)

TABLE I—(Continued)

Source	Common name	Component analyzed: Plant part (see key)	Component analyzed: Weight /1,000 g.	Oil content, % D.B.	Protein content (N×6.25), % D.B.	Fatty acid content of oil: Iodine value, Wijs	Saponification value	Refractive index $n^{40/D}$	Triene as C_{18} acid: Nonconj., %	Triene as C_{18} acid: Conj., %	Diene as C_{18} acid: Nonconj., %	Diene as C_{18} acid: Conj., %	Monoene, as oleic, %	Saturates, %	HBr uptake, as C_{18} epoxy acid, %	C=O as C_{18} acid, %	OH as C_{18} acid, %	Infrared
Rosales (cont'd)																		
Leguminosae																		
34. *Bauhinia purpurea*	Purple bauhinia	A	263.0	20	28	100	182	1.4641	0	0	48	0	13	34	1.0	-	...	Usual
35. *Calliandra eriophylla*[5]	Falsemesquite calliandra	A	18.5	16	39	82	185	1.4654	0	0	26	4.7	28	36	14	-	8	Usual
36. *Cercidium floridum*	Blue paloverde	A	222.0	16	54	100	183	1.4670	0	0	36	3.2	32	24	9.6	-	9	Usual
37. *Coumarouna alata*	Tonka bean	A	246.0	45	15	72	183	1.4572	0	0	10	0	58	27	0.3	0.3	...	Usual
38. *Erythrina* sp.	Coral-tree	A	113.6	19	42	95	176	1.4639	0	0	36	0	31	28	1.4	0.4	1	Usual
39. *Gliricidia* sp.	Madre	A	106.2	20	48	83	184	1.4620	1	0	27	0	33	34	0.4	-	...	Usual
40. *Machaerium roseseens*	.....	A	83.0	23	42	85	170	1.4635	0	0	33	0	26	33	1.0	-	...	10% *Trans*
41. *Medicago tribuloides*	Barrel medic	A	4.0	16	44	133	181	1.4674	24	0	22	0	30	20	0.8	-	3	Usual
42. *Milletia ovalifolia*	Milletia	A	221.0	37	27	100	186	1.4722	0	0	33	0	45	18	0.6	2.0	...	.....
43. *Pongamia pinnata*	Poonga oil tree	A	818.0	35	22	88	181	1.4730	1	0	19	0	56	19	0.5	-	...	.....
44. *Stylosanthes gracilis*	Pencil flower	A	2.8	17	40	110	184	1.4659	1	0	46	0	27	22	0.9	-	...	Usual
45. *Tephrosia* sp.	Tephrosia	A	30.9	10	44	85	181	1.4639	1	0.4	25	0	40	29	0.1	-	...	Usual
46. *Trifolium subterraneum*	Subterranean clover	A	7.4	15	36	110	184	1.4650	2	0	46	0	24	24	2.1	-	...	Usual
Geraniales																		
Zygophyllaceae																		
47. *Balanites aegyptiaca*	Lalob tree	A	890.0	46	22	93	186	1.4641	0	0	37	3.2	23	32	7.9	-	7	Trace ROH
Rutaceae																		
48. *Ptelea trifoliata*	Water-ash	E	11.0	46	44	179	182	1.4731	30	0	42	0	22	1	0.8	-	...	Usual
49. *Skimmia japonica*	Japanese skimmia	A	14.0	43	26	144	182	1.4695	26	0	20	0	42	8	0.7	0.4	...	Usual
Simaroubaceae																		
50. *Ailanthus altissima*	Tree-of-heaven	B	9.0	56	28	133	188	1.4677	0	0	56	0	35	4	0.5	-	...	Usual
51. *Picramnia pentandra*	Florida bitterbush	A	93.0	55	12	86	181	1.4670	1	0	1	0	93	2	0.1	2.0	1	*Trans*; ROH
Meliaceae																		
52. *Trichilia* sp.	Bitterwood	A	63.2	54	14	127	182	1.4701	(16)[1]	0	(12)[1]	0	(69)[1]	(-2)[1]	0.6	1.0	...	Many bands
Euphorbiaceae																		
53. *Croton texensis*	Skunkweed	A	7.0	26	25	165	186	1.4730	28	0	46	0	8	15	0.1	0.1	...	Usual
54. *Daphniphyllum humile*	.....	A	50.0	35	15	100	178	1.4642	1	0	28	0	53	14	0.7	-	...	Usual
55. *Mercurialis annua*	Herb-mercury	A	1.5	37	19	211	190	1.4763	70	0	8	0	7	12	0.2	-	1	Usual
56. *Phyllanthus* sp.	Leafflower	A	11.0	30	14	160	187	1.4705	35	0	21	0	30	10	0.1	0.8	0	Usual
Sapindales																		
Celastraceae																		
57. *Celastrus orbiculata*	Oriental bittersweet	A	9.0	46	22	116	245	1.4747	21[3]	0	20	0	16	36	2.3	-	...	8.0 μ band
Sapindaceae																		
58. *Koelreuteria formosana*	Flame goldenrain-tree	B	40.0	53	29	82	176	1.4643	5	0	11	0	54	25	0.2	-	0	Triple bond
59. *Sapindus mukorossi*	Chinese soapberry	B	321.0	35	31	89	186	1.4634	3	0	11	0	69	14	0.3	-	...	Usual
Balsaminaceae																		
60. *Impatiens balsamina*	Garden balsam	A	8.7	22	16	174	187	1.5026	0	0[6]	34	0	...[1]	...[1]	0.3	-	...	Parinaric acid
Rhamnales																		
Rhamnaceae																		
61. *Rhamnus cathartica*	Common buckthorn	D	13.1	25	20	166	184	1.4718	31	0	36	0	19	10	0.3	0.7	...	Usual
62. *Rhamnus davurica*	Dahurian buckthorn	D	24.4	27	20	159	185	1.4718	24	0	42	0	18	11	0.3	1.0	4	Usual
63. *Rhamnus purshiana*	Cascara sagrada	B	30.3	57	25	108	186	1.4648	3	0	28	0	55	10	0.2	-	...	Usual
Vitaceae																		
64. *Vitis vulpina*	Winter grape	A	27.6	16	10	142	181	1.4688	0	0	(56)[1]	0	(44)[1]	(-5)[1]	0	-	...	Usual
Malvales																		
Malvaceae																		
65. *Abutilon theophrasti*	Velvetweed	A	8.5	15	23	130	181	1.4683	1	0	63	0	16	16	1.0[7]	-	...	Usual
66. *Hibiscus cannabinus*	Kenaf	A	22.5	18	28	107	186	1.4645	1	0	43	1.5	28	23	4.8[7]	-	...	Usual
67. *Hibiscus moscheutos*	Rose-mallow	A	7.4	13	22	118	188	1.4680	1	0	46	0	36	13	15[7]	-	...	Band at 9.9 μ
68. *Hibiscus syriacus*	Rose-of-Sharon	F	7.3	29	29	116	188	1.4667	0	0	43	0	42	11	21[7]	-	...	Band at 9.9 μ
69. *Lavatera trimestris*	Herb tree-mallow	A	6.5	16	25	100	192	1.4664	0	0	30	3.8	42	19	12[7]	-	5	Usual; trace ROH
Sterculiaceae																		
70. *Brachychiton acerifolius*	Flame-tree	B	154.0	30	36	116	178	1.4654	1	0	46	0	34	15	11[7]	-	...	Usual
71. *Firmiana simplex*	Chinese parasol-tree	B	97.0	37	26	109	184	1.4652	1	0	39	0	40	15	16[7]	-	...	Usual
Parietales																		
Loasaceae																		
72. *Mentzelia decapetala*	Ten petal blazingstar	A	0.6	42	21	140	193	1.4689	4	0	58	0	26	8	0.6	-	1	Usual
Myrtales																		
Elaeagnaceae																		
73. *Elaeagnus angustifolia*	Russian olive	A	15.9	26	42	155	184	1.4702	14	0	48	0	32	1	0.4	-	...	Trace ROH; trace-*trans*; high free acid
Lythraceae																		
74. *Cuphea llavea* var. *miniata*	Cinnabar cuphea	A	1.0	21	17	18	272	1.4480	0	0	5	0	8	82	0.2	-	...	Trace-*trans*

(Continued)

TABLE I—(Continued)

Source	Common name	Component analyzed: Plant part (see key)	Component analyzed: Weight /1,000 g.	Oil content, % D.B.	Protein content (N×6.25), % D.B.	Iodine value, Wijs	Saponification value	Refractive index $n^{40/D}$	Fatty acid content of oil: Triene as C_{18} acid, Nonconj., %	Triene as C_{18} acid, Conj., %	Diene as C_{18} acid, Nonconj., %	Diene as C_{18} acid, Conj., %	Monoene, as oleic, %	Saturates, %	HBr uptake, as C_{18} epoxy acid, %	C=O as C_{18} acid, %	OH as C_{18} acid, %	Infrared
Myrtales (cont'd)																		
Punicaceae																		
75. *Punica granatum*	Pomegranate	A	24.0	4	8	163	183	1.5146	...[1]	62	...[1]	0	...[1]	...[1]	0	-	...	Conj. triene
Onagraceae																		
76. *Oenothera biennis*	Common evening-primrose	A	0.6	25	16	153	188	1.4702	8	0	61	0	24	3	0.3	-	...	Usual
77. *Oenothera rhombipetala*	.....	A	0.2	31	16	151	184	1.4693	5	0	69	0	13	8	0.2	-	...	Usual
Umbellales																		
Umbelliferae																		
78. *Anthriscus cerefolium*	Salad chervil	C	2.1	16	19	108	184	1.4648	0	0	30	0	59	6	0.3	-	2	Usual
79. *Daucus carota*	Carrot	E	1.7	30	30	106	162	1.4660	0	0	13	0	81	1	1.6	8/	7	Usual; Ca 15% ROH
80. *Daucus carota*	Carrot	E	0.8	26	25	162	...	1.4749	[1]	0	[1]	2.8	...[1]	...[1]	20	5	...	Many weak bands
81. *Daucus pusillus*	Southwestern carrot	E	0.8	27	20	122	130	1.4783	[1]	0	[1]	0	(136)[1]	(-40)[1]	0.5	-	...	Many bands
82. *Heracleum lanatum*	Common cow parsnip	C	5.6	18	22	87	200	1.4773	...[1]	...	...[1]	...	...[1]	...[1]	14	-	...	Many bands 7–15 μ
Ericales																		
Ericaceae																		
83. *Arctostaphylos glauca*	Great-berried manzanita	A	11.0	56	25	157	189	1.4704	25	0	33	0	32	6	0.2	-	...	Usual
Ebenales																		
Symplocaceae																		
84. *Styrax americana*	Mock-orange	A	16.6	43	19	124	173	1.4809	9	0[9]	28	0	39	16	0.6[7]	-	...	Many bands
85. *Symplocos paniculata*	Sapphireberry sweetleaf	A	17.0	52	21	115	192	1.4656	0	0	36	0	53	6	0.5	-	...	Usual
Gentianales																		
Loganiaceae																		
86. *Buddleia davidi*	Orange eye butterflybush	A	0.1	28	19	130	175	1.4684	1	0	59	0	22	13	0.2	-	...	Usual
Apocynaceae																		
87. *Apocynum cannabinum*	Hemp dogbane	A	0.6	23	29	151	184	1.4708	10	0	53	0	30	2	0.4	-	...	Usual
88. *Thevetia* sp.	.....	A	4648.0	58	16	79	181	1.4615	0	0.1	17	0	54	25	0	-	...	Usual
Asclepiadaceae																		
89. *Asclepias engelmanniana*	Milkweed	A	6.1	38	42	114	186	1.4667	2	0	34	0	53	6	0.3	8/	...	Usual
90. *Asclepias incarnata*	Swamp milkweed	A	3.6	27	31	119	181	1.4667	2	0	39	0	48	6	0.3	-	2	Usual
91. *Marsdenia edulis*	.....	B	10.0	47	27	85		1.4624	0	0	18	0	58	20	0.6	8/	...	Usual
Polemoniales																		
Boraginaceae																		
92. *Onosmodium occidentale*	Western marbleseed	B	18.5	56	36	190	...	1.4738	(46)[1]	0.1	(18)[1]	0	(35)[1]	(-3)[1]	0.2	-	...	Usual
Labiatae																		
93. *Hyptis suaveolens*	Bushmint	C	7.4	24	22	146	188	1.4696	0	0.2	77	0	6	12	0	-	...	Usual
94. *Leonurus cardiaca*	Common motherwort	C	0.4	30	22	133	180	1.4689	2	0	53	0	35	6	0.5	-	...	Usual
95. *Lycopus asper*	Bugleweed	C	0.7	25	17	155	168	1.4719	30	0	16	0	49	0	1.6	-	...	Many weak bands; high free ac
96. *Mentha arvensis*	Field mint	C	0.1	23	18	199	183	1.4752	58	0	16	0	13	9	0.2	1.0	3	Usual
97. *Mentha* sp.	Mint	C	0.6	44	25	214	...	1.4767	64	0	17	0	9	5	0.1	-	0	Usual
98. *Nepeta cataria*	Catnip	C	0.5	21	18	201	185	1.4754	57	0	18	0	12	8	0.2	-	1	Usual
99. *Pycnanthemum muticum*	Mountain mint	C	0.4	35	31	213	184	1.4773	62	0.1	14	0	19	0	0.2	-	...	Usual
Solanaceae																		
100. *Datura metel*	Hindu datura	A	14.1	19	14	110	179	1.4658	1	0	40	0	39	16	0.3	-	...	Usual
Scrophulariaceae																		
101. *Alonsoa warscewiczii*	Heartleaf mask flower	A	0.9	26	20	128	172	1.4732	(3)[1]	0.1	(36)[1]	0	(61)[1]	(-4)[1]	0.3	-	...	Usual
102. *Antirrhinum majus*	Common snapdragon	A	0.2	43	16	141	206	1.4684	0	0	69	0	17	9	0.2	-	...	Usual
103. *Nemesia suttonii*	Pouch nemesia	A	0.2	48	29	130	177	1.4638	2	0	60	0	18	16	0.8	8/	...	30% Free acid; trace ROH
104. *Paulownia tomentosa*	Royal paulownia	F	0.1	24	12	139	185	1.4690	0	0	66	0	19	9	0.2	-	...	Trace ROH
105. *Penstemon albidus*	White penstemon	A	0.6	20	15	128	140	1.4818	(1)[1]	0	(34)[1]	7.6	(58)[1]	(-4)[1]	3.4	20.0	8	Ca 30% ROH; 30% free acid
106. *Penstemon grandiflorus*	Shell-leaf penstemon	A	1.6	17	15	128	166	1.4719	0	0	49	0	42	4	1.8	-	8	Usual
107. *Veronica spicata*	Spike-speedwell	A	0.5	27	26	151	192	1.4701	2	0	70	0	21	3	0.2	-		Usual
Bignoniaceae																		
108. *Catalapa bignonioides*	Common catalpa	F	15.0	33	36	153	173	1.4884	0	28	36	0	14	18	0.6	-	3	Like eleostearic
109. *Chilopsis linearis*[5]	Desert willow	A	5.0	32	20	142	183	1.4824	0	21	28	12	14	21	0.3	-	...	Like eleostearic
Rubiales																		
Rubiaceae																		
110. *Cinchona*	Cinchona	B	0.4	16	18	115	177	1.4663	0	0	42	0	43	10	2.5	-	...	Usual
111. *Gardenia jasminoides*	Cape-jasmine	A	3.4	20	14	122	184	1.4660	0	0	50	0	35	11	0.4	1.0	...	Usual
Caprifoliaceae																		
112. *Lonicera tatarica*	Tartarian honeysuckle	A	2.5	32	21	140	184	1.4682	1	0	68	0	18	10	0.7	-	...	Trace ROH
113. *Viburnum dentatum*	Arrow-wood	A	11.0	42	17	97	180	1.4636	0	0	16	0	74	5	0	-	...	Usual

(Continued)

TABLE I—(Continued)

Source	Common name	Component analyzed: Plant part (see key)	Weight /1,000 g.	Oil content, % D.B.	Protein content (N×6.25), % D.B.	Iodine value, Wijs	Saponification value	Refractive index $n^{40/D}$	Fatty acid content of oil: Triene as C_{18} acid, Nonconj., %	Triene as C_{18} acid, Conj., %	Diene as C_{18} acid, Nonconj., %	Diene as C_{18} acid, Conj., %	Monoene, as oleic, %	Saturates, %	HBr uptake, as C_{18} epoxy acid, %	C=O as C_{18} acid, %	OH as C_{18} acid, %	Infrared
Cucurbitales																		
Cucurbitaceae																		
114. *Cayaponia* sp.		A	52.4	18	16	143		1.4919	0	34	(31)[1]	0	(-8)[1]	...[1]	0.2	-	...	30% *Cis-trans* conj.
115. *Citrullus vulgaris*[10]	Watermelon	B	32.9	52	38	132	185	1.4678	0	0	67	0	12	16	1.0	-	...	Usual
116. *Echinocystis fabacea*		B	1157.0	51	31	114	186	1.4648	1	0	48	0	28	19	0.4	-	...	Usual; trace ROH
117. *Echinocystis oregana*	Man-root	B	948.0	47	34	115	189	1.4650	1	0	49	0	26	19	0.2	-	1	Usual; trace ROH
Campanulales																		
Compositae																		
118. *Ambrosia trifida*	Giant ragweed	C	15.8	25	22	138	185	1.4684	0.2	0	67	0	18	10	0.2	1.0	0	Usual
119. *Anthemis sancti-johannis*	St. Johns camomile	C	0.6	24	18	142	182	1.4693	0.3	0	72[1]	1.2	10[1]	12	2.0	-	...	Usual
120. *Arctium minus*	Burdock	C	9.5	21	17	159	183	1.4696	0.2	0	(70)[1]	0	(35)[1]	(-10)[1]	0.5	0.3	...	*Trans*
121. *Arctotis grandis*	African daisy	B	0.8	48	36	126	192	1.4649	0.1	0	53[1]	3.1	27[1]	13[1]	7.0	-	...	Usual
122. *Aster alpinus*	Rock aster	C	1.3	23	19	161	176	1.4712	0.8	0	(58)[1]	0.8	(59)[1]	(-22)[1]	0.3	2.0	2	*Trans*
123. *Aster ericoides*	Michaelmas daisy	C	0.2	30	28	144	187	1.4684	0.4	0	66	0	26	3	0.3	1.0	...	*Trans*
124. *Boltonia asteroides*	White boltonia	C	0.1	21	19	136	186	1.4736	0.7	0.7	54	0	39	2	2.0	-	...	*Trans*
125. *Brachycome iberidifolia*	Swan River daisy	C	0.2	27	26	125	180	1.4694	0	0.2	51	5.6	25	14	14.0	-	10	ROH; *cis,trans* conj
126. *Chrysanthemum coronarium*	Garland chrysanthemum	C	1.9	19	18	142	184	1.4694	1.3	0	66	0	21	8	3	-	...	Usual
127. *Cirsium altissimum*	Plumed thistle	C	3.2	22	19	141	182	1.4691	7.6	0	55	0	23	10	0.4	1.0	...	Usual
128. *Cnicus benedictus*	Blessed thistle	C	27.5	27	18	132	177	1.4677	0	0	62	0	22	12	0.9	-	...	Usual
129. *Cynara cardunculus*	Cardoon	C	20.2	43	28	125	182	1.4670	0.6	0	58	0.9	18	18	1.5	-	...	Usual
130. *Echinacea angustifolia*	Coneflower	C	2.0	37	49	139	183	1.4685	0.5	0	64	1.0	22	8	1.0	-	3	Usual
131. *Echinops exaltatus*	Russian globe thistle	C	11.0	35	37	139	184	1.4685	0.6	0	66	2.8	14	12	7.0	-	8	Usual
132. *Encelea farinosa*	White brittlebush	C	1.1	24	23	120	180	1.4676	0	0	55	0	23	18	0.3	-	...	Usual
133. *Eupatorium rugosum*	White snakeroot	C	0.3	40	26	143	181	1.4697	0.4	0	70	0	18	8	0.2	-	2	Usual
134. *Gaillardia aristata*	Common perennial gaillardia	B	2.0	28	46	141	186	1.4708	1.0	0	65	3.6	16	10	7.0	-	5	Usual
135. *Gaillardia pulchella*	Painted gaillardia	C	1.1	18	32	130	186	1.4690	0.4	0	60	0	17	16	4.0	-	5	Usual
136. *Grindelia squarrosa*	Curlycup gumweed	C	0.7	20	14	138	186	1.4692	0.5	0	61	0	28	6	0.5	-	2	*Trans*
137. *Helenium autumnale*	Sneezeweed	C	1.0	23	25	153	202	1.4720	(1.4)[1]	0	(69)[1]	0.1	(28)[1]	(-2)[1]	1.0	1.0	...	*Trans*
138. *Helianthus annuus*[11]	Sunflower	A	40.0	49	34	110	...	1.4651	0.1	0	39	1.2	41	14	2.9	-	...	Usual
139. *Helianthus annuus*[11]	Sunflower	A	20.0	44	32	136	...	1.4676	0.3	0	68	1.6	11	15	3.1	-	...	Usual
140. *Helianthus maximiliani*	Sunflower	C	2.0	30	31	145	184	1.4704	0.5	0	73	0	13	9	0.6	-	...	Usual
141. *Helianthus petiolaris*	Prairie sunflower	C	5.0	30	19	141	187	1.4689	0.2	0	65	0	26	5	0.7	-	...	Usual
142. *Helianthus rydbergi*	Sunflower	C	5.9	30	26	137	181	1.4691	0.1	0.1	62	0	28	6	0.6	-	...	Usual
143. *Helianthus scaberrimus*	Sunflower	C	5.4	30	24	140	184	1.4699	0.2	0	67	0	21	8	0.6	-	1	Usual
144. *Helianthus tuberosus*	Jerusalem artichoke	C	2.0	26	31	145	180	1.4697	0.7	0	64	0	31	0	0.4	-	...	Usual
145. *Hulsea heterochrome*		C	0.6	22	20	125	183	1.4678	0.3	0	55	0	28	13	0.8	-	...	Usual
146. *Iva xanthifolia*	Rag sumpweed	C	0.6	23	19	146	177	1.4700	0.8	0	73	0	14	8	0.6	-	...	Usual
147. *Kuhnia glutinosa*	False boneset	C	1.0	22	33	135	179	1.4698	0.5	0	60	0	27	8	0.8	-	3	Usual
148. *Lactuca scariola*	Bitter lettuce	C	1.3	41	29	139	184	1.4695	2.2	0	63	0	22	9	0.8	-	...	Usual
149. *Liatris punctata*	Blazing star	A	32.0	29	47	127	185	1.4675	1.1	0	54	0	28	12	0.4	-	1	Usual
150. *Listris pycnostachya*	Button snakeroot	C	1.8	30	34	134	181	1.4688	0.5	0	58	0	30	7	0.3	0.4	2	Usual
151. *Ratibida columnaris*	Coneflower	C	0.6	26	27	145	186	1.4690	0.3	0	66	0	26	2	0.3	-	...	Usual
152. *Rudbeckia laciniata*	Coneflower	C	1.7	26	38	148	188	1.4699	5.8	0	60	0	26	4	0.7	-	...	Usual
153. *Solidago canadensis*	Goldenrod	C	0.1	30	25	141	184	1.4721	0.5	0	62	0	32	2	2.0	3.0	2	*Trans*
154. *Solidago serotina*	Goldenrod	C	1.5	18	14	143	148	1.4898	(0.9)[1]	0	(36)[1]	0	(84)[1]	(-26)[1]	2.0	12.0	20	ROH; many bands
155. *Tithonia speciosa*	Scarlet tithonia	C	9.5	18	21	116	175	1.4660	0.1	0	58	1.4	8	28	4	-	5	Usual
156. *Vernonia deppeana*	Ironweed	C	0.4	20	24	107	95	...	(1.1)[1]	0.1	(3.1)[1]	0	(109)[1]	...[1]	0.7	-	...	Many bands
157. *Vernonia fasciculata*	Ironweed	C	1.3	28	22	131	177	1.4700	1.2	0	56	0	29	9	5	2.0	...	Usual
158. *Viguiera laciniata*	Cutleaf goldeneye	C	0.7	13	10	135	187	1.4678	0.5	0	69	0	9	17	0.6	-	3	Usual
159. *Wyethia helenioides*		C	33.5	15	21	126	176	1.4686	0.2	0	50	2.3	34	9	6.0	-	6	Trace ROH
160. *Ximenesia encelioides*	Golden crownbeard	C	1.0	31	36	107	184	1.4654	0.3	0	27	0	64	5	0.4	-	...	Usual

[1] Method not applicable or questionable. [2] Negative carbonyl test indicated by dash. [3] 2% Tetraene. [4] 4% Tetraene. [5] Average of two similar samples. [6] 25% Conjugated tetraene. [7] Gives positive Halphen test. [8] Not tested. [9] 4% Conjugated tetraene. [10] Average of four similar samples. [11] Of eight samples analyzed, the two cited are the extremes with respect to I.V., linoleic acid, and conjugated diene. Deviations in other characteristics were slight.

Key: A—Seed. B—Seed minus seed coat. C—Pericarp plus seed. D—Endocarp plus seed. E—Seed plus part of pericarp. F—Part of seed coat removed.

recent references that we have encountered. Many plant families and genera that we listed earlier (1) appear again, but no species appears a second time unless the oil composition differs distinctly from the earlier sample.

Materials and Methods

Sample origin, sample preparation, and analytical procedures used were as previously described (1). In addition, a qualitative test for carbonyl (4) was made on the present oils. On oils positive to this test, the amount present was estimated by a colorimetric procedure (5).

Hydroxyl content was determined chemically (6) on methyl esters from selected oils, including a number indicated by infrared absorption to have significant amounts. Methyl esters were prepared by well-known transesterification (7) or diazomethane [for preparation see (8)] procedures most appropriate in consideration of functional groups present, sample quantities available, or the desire to exclude unsaponifiables. For convenience, the hydrogen bromide absorption was calculated in terms of the percentage of oxirane oxygen as apparent epoxy oleic acid although the lack of specificity of the method is now well recognized (9, 10). Gas chromatographic analyses were carried out on methyl esters from selected oils on a Burrell K-5 Kromotog with Apiezon-L and Resoflex-446 packed columns.

When more than one sample of a given species was analyzed, results were averaged unless there was some dissimilarity. The two *Daucus carota* [79, 80][3] samples differed markedly in HBr absorption, infrared absorption, and behavior on isomerization. The *Helianthus annuus* oils showed a range in composition similar to published results (2), and the two samples reported [138, 139] give the extremes in iodine value and linoleic acid content for the eight samples analyzed. The amount of conjugated diene and HBr absorption (possibly dimorphecolic acid) in the *H. annuus* did not vary greatly.

Results and Discussion

The species analyzed are listed in Table I according to orders in the plant kingdom so that familial similarities of chemical characteristics are more readily apparent.

Three species, *Zelkova serrata* [12], *Sassafras albidum* [23], and *Cuphea llavea* var. *miniata* [74], produced oils with such low iodine values that most of the acids present must be saturated. These three and *Celastrus orbiculata* [57] had high saponification values, indicating the presence of acids probably smaller than the usual C_{18} acids. Hopkins and Chisholm (11) showed that *Z. serrata* oil contains primarily capric (73%) and caprylic (8%) acids; our results are in excellent agreement. Our chromatographic analyses (Table II) of the esters of *S. albidum* and *C. llavea* show that these oils are very similar to that of *Z. serrata* but that the *C. llavea* oil contains even more capric acid. Chromatography of the esters from *Celastrus orbiculata* shows no significant amounts of short-chain acids. The high saponification value may result from esters of formic and acetic acids, such as have been reported in other *Celastrus* species (2), which would be lost in the routine preparation of the methyl esters.

[3] Numbers in brackets refer to position in Table I.

TABLE II

Gas Chromatographic Analyses of Methyl Esters from *Cuphea llavea*, *Sassafras albidum*, and *Zelkova serrata* Seed Oils

Type of acid	*C. llavea*	*S. albidium*	*Z. serrata*
Saturated	%	%	%
C_6	....	13	....
C_8	1	5	9
C_{10}	83	59	77
C_{12}	1	17	3
C_{14}	1	1	1
C_{16}	3	....	2
C_{18}	1	....	....
Monene			
C_{18}	5	5	3
Diene			
C_{18}	6	....	4

Sassafras albidum, a small tree in the family *Lauraceae*, is the source of a spicy root bark used for preparing a medicinal tea and a flavoring extract for various uses (12). *Lindera* oils in this same family have been reported to contain saturated acids 10 to 14 carbon atoms in chain length along with the corresponding monoenes (2, 11). The sassafras oil by gas chromatographic evidence contains capric, lauric, and caproic acids as major components in the order of decreasing amount.

Cuphea llavea, family *Lythraceae*, belongs to a genus of about 100 species of herbs and subshrubs native to the subtropics and warm temperate areas of North and South America. Several of the species are grown as ornamentals in greenhouses in the North and in the open in the South. The outstanding high content of capric acid makes this oil the richest known natural source of this acid. *Cuphea*, containing numerous herbaceous species, undoubtedly has higher crop potential than *Sassafras* or *Zelkova*.

Confirmation of gas chromatographic data on these oils by conventional characterization is desirable and contemplated. As in the case of *Zelkova* oil, the lower-molecular-weight saturated acids are not accompanied by their unsaturated counterparts, a point of possible significance in the biosynthetic process (11).

The oils of high iodine value (above 190) encountered among the species studied, although many are previously unreported, occur largely in families and genera—the mint and spurge groups—known to contain good drying-type oils and hence are not unexpected. Five species contained more than 55% "apparent linolenic" acid; the highest, with 70%, was *Mecurialis annua* [55], a spurge. From this maximum the content of linolenic acid ranged down to 0%.

Onosmodium occidentale [92] in the borage family is systematically closely allied to the mints; the high iodine value of its seed oil is therefore not surprising. *O. occidentale* seed is however most unusual in its exceptionally high ash content, 48.9% on a dry basis. Hand separation of kernel from hull revealed that the ash was largely in the hull, which contained 59.0% of total ash (15.2% of SiO_2). The hull as we obtained it comprised 83% of the seed as received; oil and protein analyses in Table I refer only to the kernel (3.6% ash). *Onosmodium* is a small genus made up mostly of coarse perennial herbs found on dry, gravelly, or calcareous soils throughout much of the eastern half of the United States.

Nine species produced oils containing more than 70% "apparent linoleic" acid. Oil from *Hyptis suaveolens* [93], a mint, contains the highest concentration, 77% (equivalent to 80% in the mixed acids). If the acid is found to be linoleic, *H. suaveolens* will be among the richest sources.

Exceptionally low saponification values (below 165) were obtained on seed oils from *Iris germanica* [10], *Ximenia americana* [14], *Daucus carota* [80], *Daucus pusillus* [81], *Penstemon albidus* [105], *Solidago serotina* [154], and *Vernonia deppeana* [156]. When refractive index is plotted against iodine value, points for all these oils, except possibly the last, lie off the line representing oils made up only of the common C_{18} acids (Figure 1). This deviation from the typical

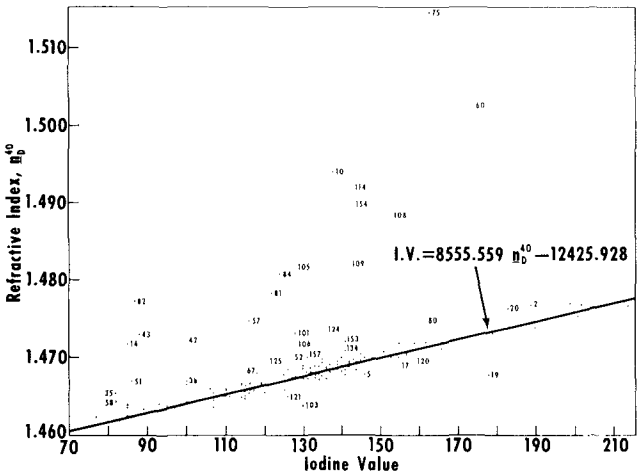

FIG. 1. Relation between iodine value and refractive index (regression line from reference 1a). Numbers on points refer to the numbered items of Table I.

behavior of common vegetable oils is sometimes ascribable to structural features, such as conjugated unsaturation, high carbonyl content, presence of essential oils, presence of apparent hydroxy acids, or excessively high content of unsaponifiables. In other cases it is due to unexplained factors that are revealed only by the presence of unidentified bands upon infrared analysis or by the failure of the usual analytical methods to provide reasonable values (*e.g.*, large negative value for the percentage of saturated acids). For example, seed oils of four species of the genus *Penstemon* have been found in our studies to contain 9 to 18% unsaponifiable matter, values much in excess of the normal. Many of the oils with lower than normal saponification values deserve more detailed chemical examination.

Conjugated trienes were found in pomegranate [75] (2), in catalpa [108] (13), in one previously unreported species of *Bignoniaceae*, and in three of *Cucurbitaceae*. One of the cucurbits, *Momordica balsamina* (balsam apple), not reported in this paper because of similarity to a previous sample (1), has been given a preliminary field trial, and it appears to be well adapted to conditions at College Station, Tex. The species has been grown both with and without trellises; its viny habit with concomitant difficulties in mechanized harvest somewhat limits its crop potential.

Of a number of species containing some conjugated diene, only *Chilopsis linearis* [109] (which also had conjugated triene) had more than 10% in its constituent acids, according to ultraviolet spectroscopy. In practically every instance where there is conjugated dienoid unsaturation, there is at least an equivalent amount of hydrogen bromide absorption in the Durbetaki titration (reported arbitrarily as apparent epoxy acid) and of hydroxyl. In selected instances lithium aluminum hydride reduction (9) confirmed that the HBr uptake is not due to true epoxy groups. This combination of conjugated diene, hydroxyl, and HBr absorption is characteristic of dimorphecolic acid (1c) and compounds with related structures. It appears probable that dimorphecolic acid is rather widespread in nature and indeed occurs in a considerable number of families other than the *Compositae*.

Among the oils reported none was found with outstandingly high percentages of HBr-absorbing components, such as *Vernonia anthelmintica* (65–70%) (14) and *Dimorphotheca aurantiaca* (*ca.* 50%) (1c). However a surprising number of the oils contain amounts from several tenths up to 20% or so of acids reactive in this titration. These undoubtedly have a number of chemically different groupings. For example, *Hibiscus moscheutos* [67] and *H. syriacus* [68] samples give a positive Halphen test and have a prominent infrared band at 9.9 μ, indicating the probable presence of acids similar to sterculic.

Twenty-nine oils gave positive qualitative tests for carbonyl, but only five contained enough to correspond to as much as 5% of a C_{18} keto acid. The types of carbonyl compounds present are unknown. In the two *Penstemon* samples [105, 106] approximately half the carbonyl occurs in the unsaponifiable fraction. Although the carbonyl-containing compounds have not been identified, the analyses provide an indication of their occurrence and amount.

Hydroxyl determinations on 48 methyl ester samples, selected on the basis of analyses on the raw oils, verified the presence of apparent hydroxy acids in all but four, in amounts ranging from 1 to 29%. Our qualitative infrared examination of the raw oils does not permit unequivocal detection of small amounts of hydroxy acids because of the presence of free acids and glyceryl hydroxyls. Failure of infrared to demonstrate the presence of hydroxyl groups in *Polanisia viscosa* [30] however is surprising, and the explanation is not evident. Gas chromatography shows the oil to be of complex composition but does not reveal known hydroxy compounds. In other instances, hydroxyl found in the esters is probably formed from epoxy groups during acid transesterification, but such a reaction cannot occur to a significant extent with *Polanisia* oil.

Figures given for apparent monoene and saturated acids, calculated by difference as they are in standard procedures, provide reliable values when usual types types of C_{18} acids are present. If unusual types of acids are present, such values may serve only as guides to oils that deviate from the norm and require further study. Infrared analysis sometimes provides a similar guide.

Among the 23 oils obviously unsuited for complete analysis by the alkali-isomerization method, three contained conjugated triene or tetraene in sufficient quantity to give erroneous results for the iodine value. Seven have many absorption bands in the infrared not associated with the common fatty acids. One, *Ximenia americana* [14], contains an acetylenic bond conjugated with an ethylenic bond (2). Two, *Podocarpus nagi* [1] and *Thalictrum revoltum* [18], probably contain a double bond too far removed from other double bonds to conjugate under the influence of heat and alkali. Such a structure has been proposed for *P. nagi* (15); gas chromatography of *T. revolutum* shows a large amount of apparent triene rather than

the diene given by the isomerization method. One, *Penstemon albidus* [105], contains sufficient carbonyl, hydroxyl, and unsaponifiables to explain the anomalous results obtained. The remainder present no obvious reasons for inapplicability of the analyses. In fact, some oils showing from −2% to −5% saturated acids may actually be suitable for the isomerization procedure because such results are not necessarily outside acceptable limits of precision of the method.

Conversely, routine application of the isomerization method to unknown oils may give results that are incorrect but not obviously so. For example, *Picramnia pentandra* [51] appears to have a reasonable, though unusual, composition. However the oil is solid well above room temperature, and the major component has chromatographic characteristics not of the usual monoenes but of stearolic acid. About 85% of the mixed methyl esters is probably from tariric acid, known to occur in other *Picramnia* species (2), but it behaves like stearolic acid in the equipment used.

Summary

Chemical screening of seed oils continues to reveal nature's diversity. This work provides leads to numerous species which warrant further research to investigate their oil and meal in greater detail, to appraise their crop potential, and to assess their practical value for providing new oilseeds.

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Direct Conversion of Lipid Components to Their Fatty Acid Methyl Esters[1]

FRANCIS E. LUDDY, R. A. BARFORD, and R. W. RIEMENSCHNEIDER,
Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania

THE APPLICATION of gas-liquid chromatography (GLC) for analysis of lipids has created the need for a convenient, quantitative method for conversion of milligram quantities of lipid components to their fatty acid methyl esters. The methyl esters are more amenable to GLC analysis than the fatty acids or their higher-molecular-weight alkyl esters.

Transesterification with methanol and catalytic amounts of sodium methylate has proved to be a rapid and effective method for conversion of glycerides to methyl esters and has been employed for many years. The reaction is substantially complete when a mixture ($1:2^{W/V}$) of glycerides and methanol (containing sodium methylate in amounts equal to about 1% of the weight of the glycerides) are refluxed for 30 min. The same procedure however does not give complete methanolysis of sterol esters and phospholipids.

Little has been published on the methanolysis of sterol esters and phospholipids. Rollet (6) reported some measure of success in the methanolysis of egg lecithin when using tin or zinc to prevent resinification. Shinowara and Brown (7) were able to get good yields of methyl esters from methanolysis of phospholipids with 5–10% of dry hydrogen chloride as catalyst and reaction time of 36 hrs. Stoffel (8) recently described a micromethod for methanolysis of lipid components with methanol containing 5% of dry hydrogen chloride and 2 hrs. of reaction time; the methyl esters were volatilized from the unsaponifiable matter and collected.

This paper describes the results of further investigation of the use of methoxides in transesterification of lipids, particularly sterol esters and phospholipids. Quantitative conversion to methyl esters was obtained with excess methanol, which contained many times the amount of sodium or potassium methoxide normally used for methanolysis of glycerides. A simple chromatographic treatment on a silicic acid column was found effective in removing free sterols and other unsaponifiable matter from the methyl ester product.

Experimental

Sources of Samples. The cholesteryl esters (Tables I and II) were synthesized by the reaction of lard fatty acid chlorides with cholesterol as described (2). The lipid components (Tables III to VI), except for soybean phospholipids, were obtained as fractions in

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[2] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.