for 15 hr. at 100° in 1 ml. of 0.5*N* sulfuric acid in a sealed tube. (Sugar standards were treated in the same way.) The solution was neutralized with Dowex-2 (HCO_3^-) and evaporated to dryness. The residue was dissolved in a small volume of water and volumes equivalent to 100, 200, 300, and 400 μg. of solid were spotted on Whatman No. 1 paper. On the same paper sugar standards were applied in amounts varying from 10 to 100 μg. The chromatogram was developed with butanol-pyridine-water (6:4:3 v/v) descending for 24 hr. (for galactose and fucose) and with ethyl acetate, acetic acid, water (3:1:3 v/v upper phase) for mannose, and arabinose determinations. The chromatograms were air-dried for 4 hr. and then dipped into a solution of aniline phthalate in aqueous butanol-ether. The papers were air-dried and then heated in an oven at 105° for 10 min. Equal areas of appropriate spots were cut out and eluted with ethanolic-HCl for 1 hr. at room temperature. The optical density of the solution was read in a spectrophotometer at 390 mμ (in the case of arabinose, 360 mμ) and compared with the appropriate standard. The following percentages were obtained:

Galactose	58–60
Arabinose	17–17.5
Fucose	11.5–12.0
Mannose	3.0

The inositol content of OSC was estimated to be about 6% by paper chromatography of a strong acid hydrolysate. These data indicate a ratio inositol:galactose:arabinose:fucose of 1:10:3:2. Failure of OSC to move on paper chromatograms is consistent with a polysaccharide of this size and preliminary sedimentation molecular weight determinations of 2600 are in good agreement with such a structure.

Further information as to the structure of OSC was obtained by mild acid hydrolysis. Forty-eight mg. of OSC were hydrolyzed for 1 hr. with 0.1 *N* oxalic acid. Serial control experiments indicated that under these conditions arabinose and fucose were liberated, but that only traces of free galactose were formed. The reaction mixture was separated by preparative chromatography on Whatman No. 1 paper with ethyl acetate, acetic acid, and water. The major spot at the origin was cut out and eluted with water. The solution was lyophilized giving 29 mg. (60.5% yield) of a white solid which contained only galactose and inositol. The galactose content determined by the anthrone procedure was 88–93%. These data indicate that OSC contains a polygalactoside unit of 9–10 molecules attached to inositol with the arabinose and fucose molecules attached to the inositol polygalactoside.

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REFERENCES

1. Carter, H. E., Celmer, W. D., Galanos, D. S., Gigg, R. H., Lands, W. E. M., Law, J. H., Mueller, K. L., Nakayama, T., Tomisawa, H. H., and Weber, E., J. Am. Oil Chemists' Soc., *35*, 335 (1958).
2. Dawson, R. M. C., Biochim. et Biophys. Acta, *14*, 374 (1954).
3. Maruo, B., and Benson, A. A., J. Biol. Chem., *234*, 254 (1959).
4. Harris, W. D., and Pranjiwar, P., J. Am. Oil Chemists' Soc., *31*, 124 (1954).
5. McKibben, J. M., and Taylor, W. E., J. Biol. Chem., *178*, 291 (1949).
6. Radin, N. A., Lavin, F. B., and Brown, J. R., J. Biol. Chem., *217*, 789 (1955).
7. Wagenknecht, A. C., Lewin, L. M., and Carter, H. E., J. Biol. Chem., *234*, 2265 (1959).
8. Hanahan, D. J., and Olley, J. N., J. Biol. Chem., *231*, 813 (1958).
9. Hanahan, D. J., Dittmer, J. C., and Warashina, E., J. Biol. Chem., *228*, 685 (1957).
10. Block, R. J., Durrum, E. L., and Zweig, G., A Manual of Paper Chromatography and Paper Electrophoresis, Academic Press, Inc., New York, 1955.
11. Carter, H. E., Celmer, W. D., Lands, W. E. M., Mueller, K. L., and Tomizawa, H. H., J. Biol. Chem., *206*, 613 (1954).
12. Zinner, H., Ann. Chem., *622*, 133 (1959).
13. Carter, H. E., Gigg, R. H., Law, J. H., Nakayama, T., and Weber, E., J. Biol. Chem., *233*, 1309 (1958). [p. 1312 $Ba(OH)_2$ hydrol.]
14. Wilson, C. M., Anal. Chem., *31*, 1199 (1959).

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Amino Acid Composition of *Lesquerella* Seed Meals

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Seed meals from 14 species of *Lesquerella,* family Cruciferae, were analyzed for 18 amino acids. Lysine and methionine contents ranged, respectively, from 331 to 440, and 72 to 94 mg. per g. of nitrogen. When compared with 9 species of *Brassica* (rape, mustard), *Lesquerella* seeds were higher in lysine and lower in methionine. Thirteen unidentified substances were detected by the ion-exchange chromatographic method used to determine amino acids.

THE GENUS *Lesquerella,* family Cruciferae, contains about 55 species (2) native chiefly to the arid parts of western North America from east central Mexico to Alberta and Saskatchewan. About one-third of the species are annuals. Representatives of *Lesquerella* also grow in limited areas of South America, Alabama, Kentucky, and Tennessee. To our knowledge, no species from this genus has ever been cultivated.

Lesquerella seed oils differ from those of other genera of Cruciferae because of their high hydroxy-acid content (3). If the nutritional quality of the co-occurring meals is high, the potential of *Lesquerella* as an industrial oilseed will be enhanced. Reported here are the analyses of 14 species for 18 amino acids by ion-exchange chromatography. The seeds were collected from the wild by botanists of USDA's Crops Research Division.

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Materials and Methods

Mature seeds (kernel with seed coat) were removed from the pods (hull) and then ground, solvent-extracted, dried, and acid-hydrolyzed as previously described (8).

Seventeen amino acids were determined on a Spinco Model MS amino acid analyzer (7). Cystine was oxidized to cysteic acid and analyzed by the method of Schram, Moore, and Bigwood (6). Constants for each amino acid were determined with standard solutions. Several runs were made to determine precision, which was about 5% (4).

Amino acid results are reported as recommended by FAO (1), in mg. per g. of nitrogen. The essential amino acid index (EAAi) was calculated according to Oser's method (5), with one exception. When the cystine : methionine or tyrosine : phenylalanine ratios exceeded those found in egg protein, only a part of either the cystine or tyrosine values was used. For example, in *L. engelmannii* only 59 mg. of cystine, which is equal to 75% of the methionine (79 mg.), instead of 134 mg. were credited because a 3 : 4 ratio is the proportion of the two in egg protein. Since tryptophan was not determined, no ratio can be assigned for it.

Results and Discussion

Data on seed analyses, distribution of nitrogenous constituents in hydrolyzates, and amount of amino acids are summarized in Tables I and II. Recoveries of nitrogen as amino acids, ammonia, humin, and unknown materials compare to those previously reported (4,8). Amino acid compositions of the various *Lesquerella* species have more similarities than differences. On the average, individual amino acids varied by only 9.2% (mean of relative standard deviations) for the different species.

Comparison of the amino acid compositions of *Lesquerella* seed meals with those of 41 other species of Cruciferae (4) show that at the 99% probability level the former are significantly higher in lysine, threonine, hydroxyproline, and proline and are lower in methionine, phenylalanine, leucine, and aspartic and glutamic acids. Isoleucine and histidine in *Lesquerella* meals are significantly lower at the 95% probability level.

When compared to 9 *Brassica* seed meals (4) the *Lesquerella* meals are significantly higher in lysine and lower in methionine, leucine, and histidine at the 99% probability level. At the 95% level *Lesquerella* meals are significantly higher in arginine and glycine and lower in cystine, phenylalanine, and isoleucine.

Unknowns in the Samples Analyzed. Thirteen unidentified peaks, usually small, appeared on the chromatograms (Table III). The largest peak, which eluted in the position of canavanine, ($R_{lysine} = 1.75$), was obtained from meal of *L. lindheimeri.* Insufficient sample prevented positive identification. The second largest peak, eluting shortly after the buffer breakthrough ($R_{methionine} = 0.971$), was obtained from meal of *L. densipila.* The third largest peak, eluting at $R_{methionine} = 0.966$, was obtained from meal of *L. lescurii.* Also obtained from this same meal was a small peak eluting at $R_{methionine} = 0.971$, apparently the same component mentioned above from meal of *L. densipila.* In addition to these peaks read at observed maximum of 570 mμ, there were three peaks read at observed maximum of 440 mμ, one of which occurred in all samples eluting at $R_{lysine} = 0.811$. Also occurring in all samples was a peak absorbing at 570 mμ and eluting at $R_{lysine} = 0.657$. These unidentified peaks probably contribute to the unknown nitrogen values given in Table I, although some nonnitrogen-containing compounds give color with ninhydrin (9). For example, levulinic acid has an observed maximum at 440 mμ, eluting about 2 hr. before aspartic acid ($R_{aspartic\ acid} = 0.60$).

Nutritional Evaluation. A slight modification of the latest method of Oser (5), described under Materials and Methods, provided a chemical evaluation for nutritional quality of the 14 species of *Lesquerella.* Recognition is given to the pitfalls of such an evaluation, which neglects such facts as biological availability of various amino acids, presence of antinutritional or antipalatability factors, and effects of type of processing on meals. Yet the close correlations that have been reported (5) between EAAi and biological value of numerous presently used plant-derived products suggest that EAAi has considerable utility for preliminary evaluation of new species.

Since the genus includes (Table II) several samples with EAAi above 70, further evaluation of *Lesquerella* meals by feeding trials appears warranted. The high lysine content suggests that these seed meals might be a good supplement for feed grains.

TABLE I

Oil and Protein Contents of *Lesquerella* Seeds and Nitrogen Distribution in Acid Hydrolyzates of the Meals

Species	Dry basis		Nitrogen distribution as % of total nitrogen			
	Oil	Protein (%N × 6.25)	Amino acids	Ammonia	Insoluble	Unknown[a]
	%	%				
L. angustifolia	26.2	25.0	73.2	12.3	5.9	8.6
L. argyraea	26.4	23.8	76.3	11.5	3.3	8.9
L. densipila	24.4	20.6	74.0	10.4	3.6	12.0
L. engelmannii	21.2	21.2	74.9	13.9	2.8	8.4
L. fendleri	28.1	22.5	82.0	12.6	5.0	0.4
L. globosa	39.4	24.4	76.1	12.9	2.3	8.7
L. gordonii	28.8	23.1	72.4	11.2	6.9	9.5
L. gracilis	32.7	24.4	74.0	12.4	2.9	10.7
L. grandiflora	37.2	19.4	75.6	12.1	2.8	9.5
L. lasiocarpa[b]	29.5	21.0	71.8	10.8	6.1	11.3
L. lescurii	28.1	21.2	74.8	11.1	3.2	10.9
L. lindheimeri	25.5	21.2	70.4	11.0	8.1	10.5
L. ovalifolia	24.0	24.4	73.2	12.8	2.2	11.8
L. pinetorum	26.6	22.5	76.2	12.7	3.5	7.6
High value	39.4	25.0	82.0	13.9	8.1	12.0
Low value	21.2	19.4	70.4	10.4	2.2	0.4
Mean	28.4	22.5	74.6	12.0	4.2	9.2
Standard deviation	5.0	1.7	2.8	1.0	1.9	2.9
Relative standard deviation, %	17.6	7.8	3.7	8.3	44.6	31.2

[a] By difference.
[b] Average of two accessions.

TABLE III

Unidentified Peaks on Chromatograms of *Lesquerella* Meal Hydrolyzates

Position	Wave length measured, mμ	H x W mg. N on col'n[a]	Species of *Lesquerella*
R_{lysine}			
= 0.657	570	1.1 to 2.9	All
= 0.811	440	Trace to 0.414	All
= 1.10	570	0.240 to 0.608	*Argyraea, densipila, gordonii, lasiocarpa,* and *lescurii*
= 1.75	570	Trace to 9.20	*Grandiflora, lasiocarpa,* and *lindheimeri*
$R_{hydroxyproline}$			
= 0.853	440	1.01	*Lasiocarpa*
= 0.884	570	0.865	*Densipila*
= 0.922	440	Trace	*Fendleri* and *gordonii*
$R_{methionine}$			
= 0.966	570	6.25	*Lescurii*
= 0.971	570	7.08 and 1.75	*Densipila* and *lescurii*
= 0.980	570	Trace	*Angustifolia* and *grandiflora*
= 0.989	570	0.642	*Lindheimeri*
= 1.04	570	Trace	*Argyraea, fendleri, gordonii* and *gracilis*
= 1.10	570	Trace	*Grandiflora*

[a] Area per unit of nitrogen in sample.

TABLE II
Amino Acid Composition of *Lesquerella* Seed Meals

Species	% N	Mg. amino acid per gram of nitrogen[a] Lysine	Methionine	Cystine	Isoleucine	Leucine	Phenylalanine	Tyrosine	Threonine	Valine
L. angustifolia	5.16	376	78	139	212	338	212	173	234[c]	284
L. argyraea	4.56	384	86	133	218	336	233	189	266	277
L. densipila	4.22	409	76	134	216	336	182	156	247	291
L. engelmannii	4.17	331	79	134	203	322	231	159	258	296
L. fendleri	4.53	415	84	111	222	363	239	186	278	299
L. globosa	6.04	440	84	128	230	376	224	183	240	306
L. gordonii	4.72	357	83	124	205	324	211	164	252	260
L. gracilis	5.42	440	82	134	217	356	206	169	238	288
L. grandiflora	4.74	378	94	133	221	364	204	170	252	299
L. lasiocarpa[e]	4.32	412	77	148	194	302	190	152	239	281
L. lescurii	4.58	415	72	134	220	343	199	158	242	309
L. lindheimeri	4.12	390	76	135	203	325	211	166	231	274
L. ovalifolia	4.72	333	91	133	212	352	228	179	249	289
L. pinetorum	4.58	361	89	123	217	363	219	181	252	318
High value	6.04	440	94	148	230	376	239	189	278	318
Low value	4.12	331	72	111	194	302	182	152	231	260
Mean	4.71	389	82	132	214	343	214	170	248	291
Standard deviation	0.53	35	6	8	9	21	17	12	13	15
Relative standard deviation, %	11.0	9.0	7.7	6.4	4.4	6.0	7.8	7.0	5.2	5.3

Species	Histidine	Arginine	Glycine	Alanine	Aspartic acid	Glutamic acid	Hydroxyproline	Proline	Serine	EAAi[b]
L. angustifolia	134	481	309	214	383	791	120	383	213	67
L. argyraea	148	444	335	269	449	771	149	374	284	71
L. densipila	134	422	266	229	381	859	139	479	228	67
L. engelmannii	151	468	317	275	391	749	222[d]	341	266	68
L. fendleri	158	491	371	281	452	856	154	417	290	73
L. globosa	132	503	279	215	373	842	99	441	177	71
L. gordonii	139	434	324	254	423	740	156	337	269	67
L. gracilis	135	471	286	223	369	768	108	438	182	69
L. grandiflora	141	391	343	272	443	880	124	423	274	70
L. lasiocarpa[e]	124	365	295	247	392	794	186	420	299	64
L. lescurii	129	441	274	223	389	856	142	488	224	68
L. lindheimeri	128	381	278	209	364	696	152	393	193	65
L. ovalifolia	147	442	312	260	426	802	108	344	231	70
L. pinetorum	144	460	342	264	463	844	108	348	246	71
High value	158	503	371	281	463	880	222	488	299	....
Low value	124	365	266	209	364	696	99	337	177	....
Mean	139	442	309	245	407	803	140	402	241	....
Standard deviation	10	41	31	26	34	55	34	50	41	....
Relative standard deviation, %	7.0	9.3	10	10	8.4	6.8	24	12	17	....

[a] To convert to g./16 g. N multiply by 0.016.
[b] Essential amino acid index, see text.
[c] Values underlined once vary ± 1 standard deviation from the mean.
[d] Values underlined twice vary ± 2 standard deviations from the mean.
[e] Average of two accessions.

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REFERENCES

1. Food and Agricultural Organization of the United Nations, "Protein Requirements," FAO Nutritional Studies No. 16, Rome, Italy, 1957.
2. Gleason, H. A., "The New Britton and Brown Illustrated Flora," vol. 2, p. 226, New York Botanical Garden, 1952.
3. Mikolajczak, K. L., Earle, F. R., and Wolff, I. A., J. Am. Oil Chemists' Soc., in press.
4. Miller, R. W., VanEtten, C. H., McGrew, Clara, and Wolff, I. A., J. Agr. Food Chem., in press.
5. Oser, B. L., in "Protein and Amino Acid Nutrition," ed. A. A. Albanese, chap. 10, Academic Press, New York, 1959.
6. Schram, E., Moore, S., and Bigwood, E. J., Biochem. J., *57*, 33 (1954).
7. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem., *30*, 1190 (1958).
8. VanEtten, C. H., Miller, R. W., Wolff, I. A., and Jones, Q., J. Agr. Food Chem., *9*, 79 (1961).
9. Zacharius, R. M., and Talley, E. A., J. Chromatog., in press.

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