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POLYUNSATURATED FATTY ACIDS FROM SEVERAL PLANT SPECIES OF THE FAMILY Boraginaceae

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Lipids from seeds of the plants Cynoglossum officinale (**1**), Echium vulgare (**2**), and Lappula squarrosa (**3**) *of the family Boraginaceae growing in the Republic of Bashkortostan were studied. Four polyunsaturated acids, linoleic (LA), γ-linolenic (GLA), α-linolenic (ALA), and stearidonic (SA), were identified among the fatty acids. The principal acids of the neutral lipids (NL) were 18:1 and 18:2 in **1**, ALA in **2** and **3**, and GLA in approximately equal amounts in all three samples. The highest amount of SA (16.8%) was found in **3**. Unsaponified components of NL samples were identified by GC/MS. Alkaloids were observed in the pulp and polar lipids.*

Keywords: γ-linolenic, α-linolenic, stearidonic acids; lipids; *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*, family Boraginaceae.

It is well known that linoleic (LA, ω-6 18:2 or 9,12-octadecadienoic) and α-linolenic (ALA, ω-3 18:3 or 9,12,15-octadecatrienoic) acids are essential polyunsaturated fatty acids (PUFA). They represent two families of PUFA and are precursors of their higher molecular-weight and more unsaturated components. The family ω-3 includes ALA; stearidonic (SA, ω-3 18:4 or 6,9,12,15-octadecatetraenoic); 5,8,11,14,17-eicosapentaenoic (EPA, ω-3 20:5); and 4,7,10,13,16,19-docosahexaenoic (DHA, ω-3 22:6) acids, the last two of which were isolated from fish fat [1].

The family ω-6 comprises LA; γ-linolenic (GLA, ω-6 18:3 or 6,9,12-octadecatrienoic); dihomo-GLA (DGLA, ω-6 20:3 or 8,11,14-eicosatrienoic); and arachidonic (AA, ω-6 20:4 or 5,8,11,14-eicosatetraenoic) acids [1]. PUFA are not synthesized in the human body but assimilated from food. The ultimate metabolites of PUFA, depending on their structure, are various prostaglandins [1, 2]. A lack of PUFA in the daily diet can lead to the development of a broad spectrum of diseases such as cardiovascular pathologies, inflammatory processes, viral infections, auto-immune diseases, and certain types of cancer [3, 4].

Prostaglandins are not stored in tissues but formed from PUFA through the action of various enzymes in response to various stimuli. However, the activity of enzymes wanes with age in man and also with pathological diseases. This is responsible for a reduction in prostaglandin biosynthesis [5]. Plants are known to be very rich in PUFA of the C_{18} series [2]. Plants of various species of the family Boraginaceae Juss. are used abroad as one of the principal sources of PUFA for preparing drugs and dietary biologically active additives. It is also recognized that ω-6 18:3 and ω-3 18:4 fatty acids (FAs) have chemotaxonomic significance for species of this family [6].

Considering this, it becomes understandable that plant oils containing these PUFA in sufficient quantities in their FAs have special biological value and bioavailability. The genera *Echium*, *Lappula*, and *Lithospermum* are the richest sources of 18:4 acid among plants of the family Boraginaceae [1].

We compared lipids from seeds of three plant species of the family Boraginaceae [*Cynoglossum officinale* L. (hound's tongue, **1**), *Echium vulgare* L. (blueweed, **2**), and *Lappula squarrosa* (Retz.) Dumort. (European stickseed, **3**)] that are widely distributed in the Republic of Bashkortostan (RB).

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TABLE 1. Fatty Acid Composition of Neutral Lipids of Seeds from *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*, % of ΣFA

Acid	*Cynoglossum officinale*	*Echium vulgare*	*Lappula squarrosa*	Acid	*Cynoglossum officinale*	*Echium vulgare*	*Lappula squarrosa*
12:0	–	0.2	–	20:0	Tr.	0.4	2.0
14:0	1.2	0.3	–	20:1	5.3	0.7	2.5
16:0	6.8	8.1	5.4	21:0	1.0	–	–
16:1	3.0	–	–	22:0	Tr.	–	–
18:0	1.6	3.4	1.9	22:1	9.0	–	0.8
18:1	30.3	15.0	14.9	$\Sigma_{sat.}$	10.6	12.4	9.3
18:2	28.1	22.2	12.6	$\Sigma_{unsat.}$	89.4	87.6	90.7
γ-18:3	**8.3**	**6.6**	**6.9**	$\Sigma_{monoen.}$	47.6	15.7	18.2
α-18:3	**3.8**	**37.8**	**36.2**	$\Sigma_{\gamma\text{-}18:3 + \alpha\text{-}18:3 + 18:4}$	13.7	49.7	59.9
18:4	**1.6**	**5.3**	**16.8**				

TABLE 2. Class Composition of Neutral Lipids of Seeds from *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*

Lipid class	Content, mass% of NL		
	Cynoglossum officinale	*Echium vulgare*	*Lappula squarrosa*
Hydrocarbons (HC)	1.3	0.7	0.5
Esters of sterols and fatty alcohols	0.9	0.4	0.2
with fatty acids (SE)	+	–	–
Triacylglycerides (TAG)	94.5	94.9	95.0
Free fatty acids (FFA)	1.1	0.6	1.5
Tocopherols	0.2	0.5	0.3
Sterols, triterpenols	1.5	0.4	0.3
Diacylglycerins	0.3	1.3	1.2
Monoacylglycerins	0.2	1.2	1.0

C. officinale is widely distributed on both sides of the Urals and is often found in forest-steppe and steppe zones in ruderal habitats on waste lands and forest borders and in thickets of steppe bushes. Large thickets in which the productivity of the dried aerial part of this species reaches 3.0 ctr/ha are sometimes formed as a result of the prolific seed production [7]. It is collected in Bashkortostan for phytotherapy needs. The lipid and FA compositions of seeds from *C. officinale* grown in Bashkir Botanical Garden were reported [8]. However, it was not clear whether the results could be extrapolated to seeds from this species from sites of natural growth and potential harvesting.

E. vulgare is a biennial plant that grows over all of the RB in forest and steppe zones on dry slopes and waste lands and near residences and roads. It is considered one of the best nectar-bearing plants, fruits in July–October, and grows over all of the RB in plantings of perennial herbs. Its potential coverage can reach 25% at sites with extensive disruption of the plant cover.

The species *L. squarrosa* is widely distributed in European Russia except for the Arctic. It grows on waste lands, disposal sites, and shoulders of roads and in fields and gardens.

Few reports of the lipid composition of seeds from *E. vulgare* and *L. squarrosa* have appeared. Studies in this area are limited to a determination of the FA compositions of seeds from these species [9, 10]. Information on the seed lipids of these plants, including those growing in the RB, is unavailable.

Seeds from *C. officinale*, *E. vulgare*, and *L. squarrosa* were collected in a ruderal steppe portion of Karmaskaly Region of the RB in the vicinity of Blue Lake.

The goal of the present work was to isolate and study all lipid groups such as neutral (NL) and polar lipids (PoL) and glyco- (GL) and phospholipids (PL).

Seeds together with pericarp were studied. The moisture content of seeds from **1**, **2**, and **3** was 9.6, 7.0, and 7.5%, respectively; the oil content per absolute dry mass (or NL content), 21.0, 26.3, and 24.1%, respectively.

TABLE 3. Fatty Acid Composition of Acyl-Containing Fractions of Neutral Lipids of Seeds from *Cynoglossum officinale* (**1**), *Echium vulgare* (**2**), and *Lappula squarrosa* (**3**), % of ΣFA

Acid	TAG			SE			FFA			DAG			MAG		
	1	**2**	**3**	**1**	**2**	**3***	**1**	**2**	**3**	**1**	**2**	**3**	**1****	**2**	**3**
12:0	–	–	–	–	–	–	–	–	1.0	–	0.9	–	–	–	–
14:0	–	–	0.3	0.8	3.6	16.2	0.8	–	4.2	0.8	1.9	–	7.2	–	3.3
15:0	–	–	–	–	4.8	–	–	–	–	–	–	–	–	5.2	1.3
16:0	6.0	8.6	5.4	9.9	12.5	4.1	12.4	23.1	9.6	5.9	22.8	13.6	14.8	21.8	12.9
16:1	–	–	–	–	–	–	–	2.9	0.5	0.9	–	4.1	–	–	–
17:0	–	–	0.7	–	2.3	30.8	–	–	1.2	–	–	–	Tr.	–	0.5
18:0	1.4	3.4	1.7	3.9	7.0	14.8	2.9	8.2	2.7	1.3	4.9	3.9	21.5	8.0	15.5
18:1	33.6	13.7	15.0	14.2	26.5	2.6	31.2	28.8	15.1	30.2	30.1	34.0	23.2	25.1	23.4
18:2	30.0	21.2	12.3	36.0	16.5	4.2	24.8	17.3	14.3	32.2	22.8	14.3	**7.4**	17.4	11.4
γ-18:3	**7.9**	**10.1**	**7.2**	**8.9**	**Tr.**	**1.6**	**4.1**	**1.8**	**5.0**	**10.3**	**1.4**	**–**	**1.0**	**6.8**	**3.9**
α-18:3	**3.8**	**34.4**	**35.3**	**5.6**	**9.6**	**14.0**	**4.4**	**13.4**	**35.6**	**3.8**	**6.1**	**4.9**	**–**	**5.1**	**19.7**
18:4	**1.8**	**8.6**	**18.1**	**1.0**	**7.5**	**1.6**	**0.8**	**–**	**7.1**	**2.0**	**1.0**	**7.2**	12.0	–	**6.3**
20:0	–	–	2.3	7.1	9.7	7.8	1.0	1.7	3.0	Tr.	8.1	16.2	–	10.6	1.8
20:1	5.7	–	0.7	2.9	–	–	6.6	–	0.3	3.8	–	–	–	–	–
21:0	–	–	–	–	–	–	–	–	–	0.9	–	–	–	–	–
22:0	–	–	0.3	3.5	–	1.2	1.5	–	–	0.9	–	–	–	–	–
22:1	9.8	–	0.7	6.2	–	–	9.5	2.8	0.4	7.0	–	1.8	–	–	–
$\Sigma_{sat.}$	**7.4**	**12.0**	**10.7**	**25.2**	**39.9**	**74.9**	**18.6**	**33.0**	**21.7**	**9.8**	**38.6**	**33.7**	**34.0**	**45.6**	**35.3**
$\Sigma_{unsat.}$	**92.6**	**88.0**	**89.3**	**74.8**	**60.1**	**25.1**	**81.4**	**67.0**	**78.3**	**90.2**	**61.4**	**66.3**	**66.0**	**54.4**	**64.7**

*Contains 1.1% 17:1 acid; in MAG of **1**, 12.9% of an unidentified acid.

A determination of the composition of the NL FAs showed (Table 1) that the samples contained four PUFA, i.e., LA, GLA, ALA, and SA. The component composition of **1** was the most varied. The total contents of saturated and unsaturated acids in the oils were practically the same. However, significant differences were seen in the monoenoic, 18:2, and 18:4 acids. The sample of **1** contained the greatest (47.6%) amount of monoenoic acids, mainly due to 18:1 and 22:1 acids. A significant amount of trienoic acids, mainly α-18:3, was found in equal ratios in samples of **2** and **3**. The α-18:3 acid is known to be a precursor in the biosynthesis of SA 18:4 [1]. SA was present in all samples. However, the greatest amount (16.8%) was observed in **3**.

NL in all samples were separated by column chromatography (CC) into component classes and were identified and determined quantitatively as described before [11] (Table 2). The samples were practically the same with respect to components and contents of individual lipid classes.

Seeds from all studied species contained insignificant amounts of PoL. The values in samples of **1–3** were 0.97, 0.34, and 0.41 (% of seed mass). This included GL and PL (0.42 and 0.55, 0.21 and 0.13, 0.11 and 0.30).

The GL classes of the samples did not differ and included monogalactosyl- and digalactosyldiacylglycerins as the main ones. In addition, an unidentified component with R_f 0.95 in the system for PoL [11] that gave a positive qualitative reaction for GL (with α-naphthol) was present.

Phosphatidylcholines, phosphatidylinositols, phosphatidylethanolamines, and phosphatidic acid were identified in the PL. We also found *lyso*-phosphatidylethanolamines in samples of **2** and **3** and *N*-acylphosphatidylethanolamines in the sample of **2** only.

Tables 3 and 4 present the FA compositions of all acyl-containing NL and PoL classes. The total composition of NL FAs was determined from the acid composition of their principal class, TAG (95%, Table 2). All other acyl-containing NL classes were minor components (Table 2) and did not have a noticeable effect on the acid composition of the TAG.

The same trends were observed in the PUFA content as in the NL content. The greatest amount of essential PUFA, as expected, was esterified with TAG.

TABLE 4. Fatty Acid Composition of Acyl-Containing Fractions of Polar Lipids of Seeds from *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*, % of ΣFA

Acid	Glycolipids			Phospholipids		
	Cynoglossum officinale	*Echium vulgare*	*Lappula squarrosa*	*Cynoglossum officinale*	*Echium vulgare*	*Lappula squarrosa*
12:0	–	2.5	0.8	–	3.6	0.7
13:0	3.6	–	–	–	–	–
14:0	3.2	1.0	2.8	7.0	7.9	2.4
15:0	1.7	2.0	4.8	1.3	–	0.3
16:0	24.2	41.5	18.0	25.4	10.2	16.8
16:1	4.0	–	0.5	3.7	–	0.5
17:0	6.9	1.9	1.3	–	–	0.4
18:0	5.5	14.8	5.2	4.6	5.5	5.1
18:1	17.9	10.5	12.4	15.3	11.2	12.4
18:2	22.4	14.3	11.2	29.6	30.8	28.4
γ-18:3	**2.7**	**1.1**	**3.3**	**7.4**	**–**	**5.7**
α-18:3	**2.4**	**3.8**	**18.7**	**2.6**	**22.7**	**17.3**
18:4	**–**	**4.3**	**8.7**	**–**	**8.1**	**4.5**
20:0	–	0.9	5.1	–	–	3.4
20:1	–	1.4	3.1	–	–	1.1
21:0	5.5	–	1.0	1.4	–	0.2
22:0	–	–	1.7	–	–	–
22:1	–	–	0.5	1.7	–	–
23:0		–	0.9	–	–	0.8
$\Sigma_{sat.}$	**50.6**	**64.6**	**41.6**	**39.7**	**27.2**	**30.1**
$\Sigma_{unsat.}$	**49.4**	**35.4**	**58.4**	**60.3**	**72.8**	**69.9**
$\Sigma_{monoen.}$	**21.9**	**11.9**	**12.9**	**20.7**	**11.2**	**14.0**
$\Sigma_{\gamma\text{-}18:3 + \alpha 18:3 + 18:4}$	**5.1**	**9.2**	**30.7**	**10.0**	**30.8**	**27.5**

TABLE 5. Composition of Free and Bound Sterols Isolated from Neutral Lipids of Seeds from *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*, % of Σsterols

Sterol	*Cynoglossum officinale*		*Echium vulgare*		*Lappula squarrosa*	
	free*	bound	free*	bound	free*	bound
Cholesterol	–	–	–	–	3.8	5.7
Campesterol	25.4	42.4	38.2	26.1	49.1	36.4
Stigmasterol	–	–	5.8	11.6	–	4.4
Fucosterol	15.4	5.6	9.4	–	4.3	5.4
β-Sitosterol	43.4	52.0	46.6	62.3	42.8	48.1

*Taraxasterol (15.8%) is present.

Table 4 shows that the GL and PL FA composition of the sample of **3** was the most varied. Practically all studied samples of PoL FAs had a rather high degree of saturation due to 16:0 acid. The principal monoenoic acid of all PoL samples was 18:1. The same trend in the content of it for both GL and PL of samples of **1–3** as for oleic acid in NL and TAG was observed (Tables 1 and 3). Thus, its amount was greatest in the sample of **1**. The same tendency persisted for GL and 18:2 acid. Its content in PL was practically the same. The total amount of PUFA (γ-18:3 + α-18:3 + 18:4) in GL (Table 4) was greatest in **3** (from **1**→**3**, 5.1 < 9.2 < 30.7%). The lowest amount of these acids in PL was found in **1** (10%). Their contents in **2** and **3** were practically the same (30.8 and 27.5%, respectively). Acid 18:4 was missing in PoL of the sample of **1**.

Lipid-soluble components in the studied samples consisted mainly of sterols located in the NL in both the free and esterified form, hydrocarbons, and tocopherols (Tables 2 and 5). They were identified by GC/MS after isolation and purification by chromatographic methods. The composition of the tocopherols was not determined. Esters were subjected to alkaline hydrolysis before extracting unsaponified compounds.

TABLE 6. Alkaloid Content in Seeds from *Cynoglossum officinale*, *Echium vulgare*, and *Lappula squarrosa*

Plant	Pulp, % of seed mass	Polar lipids, % calc. for seeds	Total content, % of seed mass
	pH 12	pH 12	
Cynoglossum officinale	0.17	0.02	0.19
Echium vulgare	0.01	0.13	0.14
Lappula squarrosa	0.03	0.03	0.06

Sterols of practically all studied samples (Table 5) contained β-sitosterol, campesterol, and fucosterol. Fucosterol was missing in bound sterols of *E. vulgare*. Stigmasterol was found in the free and bound forms in *E. vulgare* and in the bound form in *L. squarrosa.* Cholesterol was identified only in *L. squarrosa.* The principal components in all samples were campesterol and β-sitosterol (Table 5) with the latter dominating. These sterols were observed in practically equal amounts only in bound sterols of *L. squarossa*. Free sterols of *C. officinale* contained the pentacyclic triterpene alcohol (15.8%) taraxasterol.

High-molecular-weight fatty alcohols (HMFA, % of Σalcohols) were identified by GC/MS in the alcohol part of NL esters of *C. officinale*. The amounts were 16:0 (3.8), 18:0 (28.0), 19:0 (16.1), and 23:0 (52.1).

Hydrocarbons (HC) were identified by GC/MS (% of ΣHC) in *C. officinale*: 14:0 (5.1), 15:0 (2.8), *iso*-15:0 (8.0), 16:0 (5.8), *iso*-16:0 (8.0), 17:0 (2.9), 17:1 (4.7), 18:0 (5.1), *iso*-18:0 (2.9), 19:0 (6.5), 20:1 (3.6), *iso*-30:6 (squalene, 44.6); in *E. vulgare*: 17:0 (17.9), 20:0 (7.1), 23:0 (10.7), 24:0 (10.5), 25:0 (9.3), 26:0 (8.7), 27:0 (6.4), 28:0 (3.7), *iso*-30:6 (squalene, 25.7); and in *L. squarrosa*: 19:0 (4.8), 20:0 (8.5), 24:0 (14.0), 25:0 (21.0), 26:0 (19.0), 27:0 (16.1), 28:0 (10.1), *iso*-30:6 (squalene, 6.5).

According to Table 2 and the data presented above, the greatest amount and largest variety of HC (1.3%) occurred in the NL of **1**. The HC components of samples of **2** and **3** were similar. Squalene was identified in HC of all samples with the greatest content in *C. officinale*.

Only small differences in the compositions of NL, PoL, and their FAs were observed in seed lipids of *C. officinale* growing naturally and in those reported earlier [8] (Tables 1–3). This could be explained by an insignificant effect of the habitat and climatic conditions in the RB on the principal groups of reserve lipids. However, this did not apply to unsaponified components of *C. officinale* seeds (Table 5). Tocopherols were detected in unsaponified components of naturally growing plants (Table 2). The composition of the HMFA was less varied with 23:0 alcohol dominating. Campesterol, fucosterol, and the triterpene pentacyclic alcohol taraxasterol were identified in the sterol mixture in addition to β-sitosterol (Table 5). The HC were missing dienoic, trienoic, tetraenoic, and pentaenoic components of the 20–32 series [8]. However, alkanes of lower molecular weight of the 14:0–19:0 series; *iso*-alkanes 15:0, 16:0, and 18:0; and the isoprenoid HC squalene (30:6) were found.

Several species of the family Boraginaceae are known to contain toxic pyrrolizidine alkaloids that can occur in various organs, including seeds [12]. We determined their contents in NL, PoL, and pulp remaining after removal of them (Table 6). Alkaloids were not found in NL of all samples.

Thus, seed lipids of *C. officinale*, *E. vulgare*, and *L. squarrosa* of the family Boraginaceae growing in the RB were studied. The maximum amount of tetraenoic acid 18:4 occurred in seeds from *L squarrosa*. This acid was concentrated in the NL, namely, the TAG. The greatest total contents of trienoic acids ($\Sigma_{\gamma\text{-}18:3+\alpha\text{-}18:3}$) were found in these same lipid classes of seeds from *E. vulgare* and *L. squarrosa*; of monoenoic acid 18:1, in *C. officinale*. Alkaloids were missing in NL of all samples. Considering the wide distribution of *E. vulgare* and *L. squarrosa* throughout the RB, NL of seeds from these plants can provide a plant source of PUFA. The cultivation (introduction) of these plants would enable them to be used on an industrial scale.

EXPERIMENTAL

Mass spectra were obtained using a computerized GC/MS system including an HP 5890 chromatograph with an HP 5972A mass-selective detector. The analysis conditions were Ultra-250 quartz capillary column (250 m × 0.2 mm, bonded phase 5% $PhMeSiO_2$), initial temperature 30°C, heating rate 10°C/min, final temperature 300°C, He carrier gas flow rate 1 mL/min, vaporizer temperature 305°C. Spectra were scanned at the rate of 1 spectrum per second over scan range 39–500 amu. Data were processed using ChemStation HPMS. Mass spectra were interpreted using a library database based on spectrum–structure correlations and patterns and features of fragmentation of triterpene compounds as described before [11]. The reliability index Q for all compounds studied using this method was in the range 90–99%.

GC analysis of FA methyl esters was carried out on a GC-2014 chromatograph (Shimadzu) using an Omegawax^{TM} 250 capillary column (30.0 m × 0.25 mm) with polyethyleneglycol L stationary phase (0.25 μm), column temperature 205°C, vaporizer temperature 250°C, detector temperature 260°C, and He carrier gas at flow rate 30 mL/min.

The compositions of GL and PL FAs were determined after multiple and thorough purifications of their methyl esters from accompanying components by CC and PTLC. The isolation, separation, and identification of lipids were performed as described previously [11].

Tocopherols were detected by spraying TLC plates with a solution (0.5%) of *o*-phenanthroline followed by an alcohol solution (0.2%) of $FeCl_3$ to produce red spots on a yellow background. The model sample was hydrolyzed pharmacopoeic tocopherol acetate [13].

Isolation of Alkaloids from NL and PoL. The presence of alkaloids in NL and PoL was determined beforehand by a qualitative reaction with Dragendorff's solution. Alkaloids were detected in PL by Vaskovskii and Dragendorff's solutions.

Solutions of PoL and NL in $CHCl_3$ were treated (3×) with H_2SO_4 solution (1%): 1 × 20 mL; 2 × 10 mL; 3 × 10 mL. The last acid extract was checked for the presence of alkaloids using silicotungstic acid. The combined extracts were adjusted using NH_4OH to pH 12 and extracted (3×) by $CHCl_3$. The $CHCl_3$ extracts were combined and dried over Na_2SO_4. The $CHCl_3$ was evaporated first in a rotary evaporator and then to dryness using a vacuum pump. The alkaloid content was determined gravimetrically.

Because acid and base extractions were used to separate alkaloids from lipids (NL and PoL) and these extractions could lead to hydrolysis of the lipids, a part of the $CHCl_3$ solutions of these lipids was taken to determine the alkaloid content. The results were calculated from the PoL content in the seeds.

Isolation of Alkaloids from Pulp. The pulp remaining after extraction of NL and PoL was extracted by acetone:H_2O (8:2). The aqueous acetone extract was evaporated until the smell of acetone was practically gone and adjusted to pH 2 using H_2SO_4 solution (5%). Non-alkaloidal impurities were removed from the alkaloid sulfate salts by extraction with $CHCl_3$ (3×). Then, the acidic extracts were made basic with KOH until the pH was 12 and extracted with $CHCl_3$ for complete removal of the alkaloids. The $CHCl_3$ extracts were worked up as described above.

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