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Fatty acids of some algae from the Bohai Sea

Xiancui Li*, Xiao Fan, Lijun Han, Qingxiang Lou

Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China

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Abstract

The fatty acid compositions of 22 species of marine macrophytes, belonging to the Ceramiales, Cryptonemiales, Nemalionales, Laminariales, Chordariales, Scytosiphonales, Desmarestiales, Dictyosiphonales, Fucales, Dictyotales and Ulvales and collected from the Bohai Sea, were determined by capillary gas chromatography. The contents of polyunsaturated fatty acids (FAs) in the Bohai Sea algae, in comparison with the same species from the Yellow Sea were found to be lower. Red algae had relatively high levels of the acids 16:0, 18:1(n-7), 18:1(n-9), 20:5(n-3) and 20:4(n-6), and those examined were rich in C_{20} PUFAs, these chiefly being arachidonic and eicosapentaenoic acids. The major FAs encountered in the Phaeophyta were 14:0, 16:0, 18:1(n-9), 18:2(n-6), 18:3(n-3), 18:4(n-3), 20:4(n-6) and 20:5(n-3). C_{18}PUFAs are of greater abundance in the brown algae than in the red algae examined. All three green algae from the Ulvales had similar fatty acid patterns with major components, 16:0, 16:4(n-3), 18:1(n-7), 18:2(n-6), 18:3(n-3), and 18:4(n-3). They contained 16:3(n-3) and more 16:4(n-3), were rich in C_{18}PUFAs, chiefly 18:3(n-3) and 18:4(n-3) and had 18:1(n-7)/18:1(n-9) ratios higher than 1.

Keywords: Rhodophyceae; Phaeophyceae; Chlorophyceae; Algae; Fatty acids; Chemotaxonomy

1. Introduction

In recent years, fatty acids (FAs) in marine algae have aroused considerable interest among researchers. This is because marine plants can produce C_{18} and C_{20} polyunsaturated fatty acids (PUFAs; Kayama et al., 1989). These fatty acids are essential for nutrition of many animals, including humans (Uki et al., 1986), and are of interest in biotechnology, in food chain studies and in cosmetics (Servel et al., 1994). Fatty acids of marine macrophytes have also attracted the attention of chemotaxonomists. FA compositions of red, brown and green macrophytic algae, belonging to different taxonomic classes, orders or families and genera, have distinguishing features of taxonomic value (Miralles et al., 1990; Aknin et al., 1992; Khotimchenko, 1993, 1995). The Bohai Sea is rich in algae, in terms of both biomass and algal species; however there is no data on the fatty acids from this area. Hence, we analysed for fatty acids in 22 species belonging to the three main divisions of marine macrophytes collected from the Bohai Sea. Fatty acid compositions from each species are reported since such data are essential in order to identify distinguishing taxonomic features.

2. Results and discussion

The 22 species of algae from the Bohai Sea and their lipid contents are listed in Table 1, where as summarised therein the total lipid contents vary with algal species. The algae were divided into three groups in accordance with their total lipid contents. The first group included *Laurencia okamurai*, *Scytosiphon lomentarius*, *Desmarestia viridis* and *Dictyopteris divaricata*, in which the total lipid contents were more than 20 mg/g; and *Ceramium kondoi*, *Polysiphonia urceolata*, *Rhodomela confervoides*, *Undaria pinnatifida*, *Myelophycus simplex*, *Punctaria plantaginea*, *Sargassum kjellmanianum*, *Sargassum thunbergii* and *Ulva pertusa* belonged to the second group, in which the total lipid contents were between 10 and 20 mg/g; the total lipid contents of the remaining algae belonging to the third group were less than 10 mg/g. The results in Table 1 thus may reflect the different capability of accumulating lipids.

* Corresponding author. Fax: +86-532-2893549.
E-mail address: lixiancui@sina.com (X. Li).

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2.1. Rhodophyta

Red algal species belonging to three orders Ceramiales, Cryptonemiales and Nemalionales from the Bohai Sea were examined for their total fatty acid compositions.

Table 1
Total lipid contents in algae from the Bohai Sea

Division	Fresh weight (mg/g)
RHODOPHYCOTA	
RHODOPHYCEAE	
Ceramiales	
Ceramiaceae	
1. *Ceramium boydenii* Gepp	5.1
2. *Ceramium kondoi* Yendo	16.8
Rhodomelaceae	
3. *Laurencia okamurai* Okam.	24.1
4. *Polysiphonia urceolata* (Lightf.) Grev.	15.1
5. *Rhodomela confervoides* (Huds.) Silva	14.3
Cryptonemiales	
Corallinaceae	
6. *Corallina pilulifera* Post. et Rupr.	4.8
Dumontiaceae	
7. *Hyalosiphonia caespitosa* Okam.	9.6
Gloiosiphoniaceae	
8. *Gloiosiphonia capillaris* (Huds.) Carm.	5.7
Nemalionales	
Gelidiaceae	
9. *Gelidium amansii* Lamour.	6.5
CHROMOPHYCOTA	
PHAEOPHYCEAE	
Laminariales	
Alariaceae	
10. *Undaria pinnatifida* (Harv.) Sur.	10.9
Chordariales	
Corynophlaeaceae	
11. *Leathesia difforme* (L.) Aresh.	2.5
Scytosiphonales	
Scytosiphonaceae	
12. *Colpomenia sinuosa* (Roth) Derbes et Solier	5.6
13. *Scytosiphon lomentarius* (Lyngb.) J.Ag.	21.8
Desmarestiales	
Desmarestiaceae	
14. *Desmarestia viridis* (Muller) Lamx.	21.9
Dictyosiphonales	
Punctariaceae	
15. *Myelophycus simplex* (Harv.) Papenf.	16.0
16. *Punctaria plantaginea* (Roth) Grev.	11.4
Fucales	
Sagassaceae	
17. *Sargassum kjellmanianum* Yendo	17.3
18. *Sargassum thunbergii* (Mert.) O'Kuntze	18.8
Dictyotales	
Dictyotaceae	
19. *Dictyopteris divaricata* (Okam.) Okam.	23.7
CHLOROPHYCOTA	
CHLOROPHYCEAE	
Ulvales	
Ulvaceae	
20. *Enteromorpha intestinalis* (L.) Link	7.0
21. *Ulva lactuca* L.	9.2
22. *Ulva pertusa* Kjellm.	11.7

The total fatty acid composition of the nine Rhodophyta species (Table 2) has a distribution characteristic of the phylum (Jamieson and Reid, 1972) with high relative levels of the acids 16:0, 18:1(n-7), 18:1(n-9), 20:5(n-3) and 20:4(n-6). C_{18} polyunsaturated fatty acids (C_{18}PUFAs) are also present in significant amounts. Each red algal species examined contained significant levels of mono-unsaturated fatty acids (Table 2) chiefly 18:1(n-7) and 18:1(n-9). 18:1(n-7) was the predominant mono-unsaturated fatty acid in *Polysiphonia urceolata*. Red algae examined were also rich in C_{20} PUFAs, these being chiefly arachidonic [20:4(n-6)] and eicosapentaenoic [20:5(n-3)] acids except for *Gloiosiphonia capillaris*. *Polysiphonia urceolata* and *Corallina pilulifera* had the highest percentage of 20:5(n-3), 37.5 and 31.5%, respectively. *Gelidium amansii* accumulated 20:4(n-6) 29.4%, which greatly exceeds that of other red algal species examined. *Rhodomela confervoides* had the highest content of C_{18}PUFAs 19.2% among the red algae. Of the red algae examined, the fatty acid patterns of *Gloiosiphonia capillaris* was different from the other red algae, 16:0 predominated among its fatty acids, the PUFA content was lower than the rest of the red algae, and the total

Table 2
Fatty acid compositions of Rhodophyta (% total fatty acid content)

Fatty acid	1[a]	2	3	4	5	6	7	8	9
14:0	7.0	5.5	3.6	2.6	3.0	1.8	5.2	8.5	3.7
14:1(n-5)	0.1	0.3	0.8	0.1	0.1	0.1	0.4	0.2	0.1
Iso-15:0	0.1	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.1
Aiso-15:0	0.1	0.0	Tr.	0.1	0.0	0.1	0.4	0.1	0.1
15:0	0.71	0.6	0.4	0.3	0.3	0.5	0.6	0.6	0.6
16:0	42.1	39.2	37.0	27.8	21.9	32.6	40.0	57.1	33.7
16:1(n-7)	7.0	7.1	8.9	2.4	2.4	3.0	7.0	3.9	3.6
16:3(n-3)	0.2	0.3	0.1	0.1	0.3	0.2	0.5	0.3	0.2
16:4(n-3)	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0
i-17:0	0.4	0.6	0.2	0.6	0.4	0.5	0.9	0.7	0.4
17:0	0.1	0.0	0.1	0.0	0.4	1.0	0.0	0.0	0.4
18:0	1.5	1.0	0.8	1.5	0.9	1.9	1.3	4.1	1.3
18:1(n-9)	7.6	9.4	7.3	1.5	6.6	4.1	4.9	9.3	4.5
18:1(n-7)	3.6	2.7	4.2	7.1	6.2	4.7	5.7	4.2	2.6
18:2(n-6)	1.6	1.9	1.4	3.4	3.1	2.5	3.4	1.8	1.5
18:3(n-6)	0.1	0.4	0.3	0.4	0.5	0.2	0.5	0.1	0.2
18:3(n-3)	0.2	0.3	0.6	0.1	5.8	2.1	1.2	1.0	1.1
18:4(n-3)	0.3	0.4	1.3	0.2	9.8	1.5	2.3	0.5	1.5
20:2(n-6)	0.7	0.6	0.0	0.3	0.0	0.4	0.0	0.0	0.0
20:3(n-6)	1.3	1.2	0.0	0.5	0.0	0.3	0.0	0.0	0.3
20:4(n-6)	6.8	6.8	8.2	8.7	11.9	6.6	9.1	1.0	29.4
20:4(n-3)	0.1	0.0	0.0	0.1	0.6	0.2	0.0	0.0	0.0
20:5(n-3)	16.8	20.1	23.4	37.5	24.8	31.5	12.5	3.6	13.3
Other[b]	0.5	0.3	0.1	2.5	0.4	1.6	0.1	0.2	0.2
20:5/20:4	2.5	3.0	2.9	4.3	2.1	4.8	1.4	3.6	0.45
(n-3)PUFA	17.6	21.1	25.4	40.1	41.3	35.8	16.8	5.4	16.1
(n-6)PUFA	10.5	10.9	9.9	13.7	15.5	10.0	13.0	2.9	31.4
C_{18}PUFA	2.2	3.0	3.6	4.1	19.2	6.3	7.4	3.4	4.3
C_{20}PUFA	25.7	28.7	31.6	47.1	37.3	39.0	21.5	4.6	43.0

[a] Numbers designate algal species given in Table 1.

[b] Other+: 12:0, 20:1(n-11), 20:1(n-9), 22:1(n-11), 22:4(n-6), and 22:5 (n-3).

PUFAs were only 8.0%. *Ceramium boydenii* and *Ceramium kondoi* have similar fatty acid patterns; they contained 20:3(n-6) in 1.3 and 1.2%, respectively, more than any other red algae examined, moreover, the high level of 20:3(n-6) was the distinguishing characteristic of Ceramiales from the other red algae examined. Comparative analysis of fatty acids of *Rhodomela confervoides* with the same species from the Yellow Sea (Vaskovsky et al., 1996) gave an interesting observation: the species from Bohai Sea is richer in 20:4(n-6) and lower in 20:5(n-3).

2.2. Phaeophyta

We examined the fatty acid composition of 10 brown algal species belonging to the orders Laminariales, Chordariales, Scytosiphonales, Desmarestiales, Dictyosiphonales, Fucales, Dictyotales from the Bohai Sea (Table 3). Despite the marked morphological differences, which exist between the brown algal species, they have similar fatty acid patterns. The major FAs encountered in the Phaeophyta were 14:0, 16:0, 18:1(n-9), C_{18}PUFAs [18:2 (n-6), 18:3(n-3), 18:4(n-3)] and C_{20} PUFA [20:4(n-6) and 20:5(n-3)], which is in agreement with analyses of other representatives of this phylum (Chuecas and Riley, 1966; Jamieson and Reid, 1972). Polyenoic C_{18} and C_{20} fatty acids predominated in all species and their contents varied from 11.7 to 69.1% of the total FAs. This is typical of all brown algae (Vaskovsky et al., 1996) and distinguishes representatives of the Phaeophyta from members of the Rhodophyta and Chlorophyta.

Comparative analysis of major PUFA distribution in brown algae showed that the Phaeophyta might tentatively be divided into three groups. The first group, which includes *Desmarestia viridis*, *Punctaria plantaginea* and *Dictyopteris divaricata* had C_{18} PUFAs as the most abundant polyenoic FAs. The second group, which includes *Leathesia difforme*, *Scytosiphon lomentarius* and *Sargassum kjellmanianum*, contained C_{20} polyenoic FAs in a greater proportion than C_{18} PUFAs. The remaining algal species, which had nearly the same C_{18} and C_{20} PUFA content, were placed in the third group.

Polyunsaturated FAs belong to two main series, (n-3) and (n-6). PUFAs of the (n-3) series were abundant in most of the Phaeophyta, and the highest content 52.1% was found in *Myelophycus simplex*. There are some algal species with equal (n-3) and (n-6) PUFA contents, e.g. *Undaria pinnatifida*, *Leathesia difforme*, *Desmarestia viridis* and (n-6) PUFAs dominated in remaining species. Two representatives of the Fucaceae had different fatty acid patterns, *Sargassum kjellmanianum* was rich in (n-6) PUFAs and *Sargassum thunbergii* rich in (n-3) PUFAs. The content of arachidonic acid in *Sargassum kjellmanianum* exceeded that of eicosapentaenoic acid,

Table 3
Fatty acid compositions of Phaeophyta (% total fatty acid content)

Fatty acid	10[a]	11	12	13	14	15	16	17	18	19
14:0	3.6	8.8	7.5	4.2	14.4	2.8	8.9	4.0	4.0	6.8
14:1(n-5)	0.1	0.3	0.2	0.1	0.2	0.1	0.2	tr.	0.1	0.0
Iso-15:0	0.0	0.1	0.1	0	0.15	0.1	0.2	0.1	0.0	0.0
Aiso-15:0	0.0	0.1	0.2	0	0.14	0.1	0.3	0.0	0.0	0.0
15:0	0.3	0.4	0.7	0.1	0.57	0.33	0.9	0.3	0.3	0.5
16:0	23.9	32.3	26.4	14.4	44.6	15.3	38.7	21.1	22.0	19.7
16:1(n-7)	1.2	2.2	2.83	1.0	2.2	2.1	4.26	2.5	3.1	1.3
16:3(n-3)	0.2	0.4	0.5	0.1	0.0	0.2	0.4	0.1	0.2	0.1
i-17:0	0.8	0.3	1.0	0.9	0.9	0.6	1.7	0.5	0.9	0.8
17:0	0.1	0.0	0.2	0.0	0.0	0.0	0.3	0.6	0.5	0.0
18:0	1.7	1.8	1.6	0.4	6.8	0.5	3.1	1.3	0.8	1.4
18:1(n-9)	9.7	28.5	13.6	9.0	10.9	8.8	18.0	7.9	6.2	11.8
18:2(n-6)	9.9	4.7	5.1	5.6	3.7	4.3	3.8	15.0	5.0	7.5
18:3(n-6)	0.5	0.2	0.7	1.1	0.0	0.8	0.4	1.2	0.7	0.3
18:3(n-3)	5.8	1.7	3.6	6.2	2.1	7.0	3.1	4.6	9.8	9.5
18:4(n-3)	10.8	2.2	9.6	18.8	1.4	20.1	3.6	4.4	11.6	14.0
20:1(n-11)	0.8	0.7	0.5	0.2	3.1	0.0	1.3	0.4	0.1	0.0
20:3(n-6)	0.8	0.0	0.0	0.4	0.0	0.0	0.1	3.2	0.6	0.7
20:4(n-6)	15.5	5.5	5.1	12.0	2.7	7.6	2.4	21.0	10.2	9.6
20:4(n-3)	1.5	0.0	0.0	0.8	0.0	1.0	0.0	1.0	0.9	0.7
20:5(n-3)	12.5	8.4	12.6	24.2	1.8	23.8	4.7	5.3	17.5	9.8
Other[b]	0.1	0.1	5.9	0.1	2.5	3.6	0.4	3.2	2.1	1.4
(n-3)PUFA	30.8	12.7	26.3	50.1	5.3	52.1	12.1	15.4	40.0	34.1
(n-6)PUFA	26.7	10.4	10.9	19.1	6.4	12.7	6.7	40.4	16.5	18.1
C_{18}PUFA	27.0	8.8	19.0	31.7	7.2	32.2	10.9	25.2	27.1	31.3
C_{20}PUFA	30.3	13.9	17.7	37.4	4.5	32.4	7.2	30.8	29.2	20.8

[a] Numbers designate algal species given in Table 1.
[b] Other +: 12:0, 16:1(n-5), 16:4(n-3), 18:1(n-11), 18:1(n-7), 20:1(n-7), 20:2(n-6), and 22:1 (n-11).

on the contrary, the content of eicosapentaenoic acid in *Sargassum thunbergii* exceeded that of arachidonic acid.

Oleic acid 18:1 is a group of main FAs in brown algae. It is a major unsaturated FA in a few algal species from different order. *Leathesia difforme* was rich in 18:1(n-9) content, which made up 28.5% of the total FAs. Among the brown algal species analysed, *Desmarestia viridis* was unique in having fatty acid patterns with low PUFAs and more saturated fatty acids, and this feature was not found in other members of the Phaeophyta from Bohai Sea.

Colpomenia sinuosa from the Yellow Sea had 16:0 (14.2%), 7.7% of 18:1(n-9), 15.6% of 18:4(n-3) and 21.9% of 20:5(n-3) (Vaskovsky et al., 1996). The same alga from the Bohai Sea had more 16:0 (26.4%) and 18:1(n-9) (13.6%), and less 18:4(n-3)(9.6%) and 20:5(n-3) (12.6%) than that from the Yellow Sea.

2.3. Chlorophyta

The fatty acid composition from the Ulvales, three representative of the Chlorophyta, is given in Table 4. The species from the Bohai Sea had a green algal feature with higher levels of C_{16} and C_{18} PUFAs (Dembitsky et al., 1991) than that of red and brown algae. All three species had similar fatty acid patterns with major components, 16:0, 16:4(n-3), 18:1(n-7), 18:2(n-6), 18:3(n-3), and 18:4(n-3). They contained 16:3(n-3) and more 16:4(n-3), and were rich in C_{18}PUFAs, chiefly 18:3(n-3) and 18:4(n-3) and had 18:1(n-7)/18:1(n-9) ratios high than 1. The green algae examined contained C_{20}PUFAs (arachidonic acid and eicosapentaenoic acid), but the content of arachidonic acid and eicosapentaenoic acid was lower than that of the red and brown algae (Tables 2 and 3). The range of C_{20}PUFAs content was from 2.1 to 6.6% of total fatty acids. The high level of 18:3(n-3) has been regarded as a characteristic of this phylum. The abundance of 18:4(n-3) found in *Ulva lactuca* and *Ulva pertusa*, was previously reported in these two algae (Jamieson and Reid, 1972), but the high levels of 16:4(n-3) may prove taxonomically useful as it is in trace amounts in the other phyla. 18:1(n-7) has previously been identified in algae in only small quantity (Hitchcock and Nichols, 1971).

The monounsaturated fatty acid 18:1(n-7) has particular taxonomic value in Chlorophyta species (Johns et al., 1979). Jamieson and Reid (1972) reported the major 18:1 isomer as 18:1(n-9) in green algae collected from Scotland, and Ackman and McLachlan (1977) found 18:1(n-7) as the major 18:1 isomer in the Chlorophyta: our result was in agreement with the later (Ackman and McLachlan, 1977).

Table 4
Fatty acid compositions of Chlorophyta (% total fatty acid content)

Fatty acid	20[a]	21	22
14:0	1.7	1.1	0.9
14:1(n-5)	0.1	0.1	0.4
15:0	0.1	0.1	0.2
16:0	22.7	24.8	24.7
16:1(n-9)	0.4	0.4	0.0
16:1(n-7)	0.5	0.4	1.0
16:3(n-3)	0.2	0.2	0.2
16:4(n-3)	9.8	11.7	9.7
i-17:0	1.3	1.6	1.4
Ai-17:0	0.6	0.5	0.0
17:1(n-9)	3.7	2.3	1.8
18:0	0.3	0.3	0.8
18:1(n-9)	1.1	0.5	0.0
18:1(n-7)	6.2	7.0	9.8
18:2(n-6)	12.2	4.6	6.0
18:3(n-6)	0.6	0.8	0.8
18:3(n-3)	27.2	20.5	21.9
18:4(n-3)	3.1	17.9	13.2
20:4(n-6)	0.5	0.0	0.6
20:4(n-3)	0.8	0.8	0.5
20:5(n-3)	4.6	1.3	2.0
22:5(n-3)	0.7	2.8	2.4
Other[b]	1.0	0.1	1.1
18:1(n-7)/18:1(n-9)	5.6	14	>9.8
(n-3)PUFA	46.4	55.2	49.9
(n-6)PUFA	14.0	5.4	7.6
C_{16}PUFA	10.0	11.9	9.9
C_{18}PUFA	43.1	43.8	41.9
C_{20}PUFA	6.6	2.1	3.1

[a] Numbers designate algal species given in Table 1.
[b] Other+: 12:0, 20:1(n-9), 20:3(n-6), 22:0, 22:1(n-9), 22:4(n-6).

3. Conclusion

Studies of macroalgae from the Bohai Sea indicated that these algae have fatty acid patterns typical of red, brown and green algae from other regions. In general, red algae from the Bohai Sea contained high levels of C_{20}PUFAs, primarily that of 20:5(n-3) and 20:4(n-6): The main difference in the fatty acid compositions between red and brown algae was that the latter were richer in C_{18}PUFAs, especially in 18:4(n-3). Of the algae, green algae had the highest level of C_{18}PUFAs and the lowest in C_{20}PUFAs. Hence, fatty acid compositions may provide a chemotaxonomic basis for macroalgae.

Our results are also of applied value. Algal lipids from the Bohai Sea are rich in the (n-3) and (n-6) series of PUFAs, which are considered essential fatty acids for animals, including humans; for example, linoleic acid, linolenic acid and arachidonic acid serve an important function in growth and protection of the skin, and 20:4(n-6), 20:5(n-3), 20:3(n-6) have high biological activity. *Polysiphonia urceolata* and *Gelidium amansii* may be potential sources of 20:5(n-3) and 20:4(n-6), respectively, and algae belonging to Ulvales may serve as sources of 18:3(n-3) and 18:4(n-3).

4. Experimental

Algal species studied were collected in August on the coast of the Bohai Sea near Weihai. Fresh algae were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles, and then heated for 2 min in boiling H_2O to inactivate enzymes, dried and ground to powder in a mortar using a pestle. Lipids were extracted immediately by the method of Bligh-Dyer (Bligh and Dyer, 1959). Ground tissue was extracted with chloroform:methanol (1:2), and the residue was extracted three times with small portions of chloroform:methanol (1:1). Extracts were filtered and mixed with $CHCl_3$ and water for phase separations and then the organic phase was collected and evaporated to dryness in vacuo and the total lipid content was weighed.

Fatty acids were converted to methyl esters by transmethylation of lipid samples (4–5 mg) using 0.5 ml 1% NaOH in MeOH, followed by heating for 15 min at 55 °C, adding 1.0 ml 5% HCl in MeOH and heating for 15 min at 55 °C (Carreau and Dubacq, 1978), then adding 0.5 ml H_2O. Fatty acid methyl esters (FAMEs) were extracted by adding hexane (3×2 ml), and the organic phase was evaporated to dry under reduced pressure. FAMEs were purified by TLC using benzene as solvent and analysed by FID–GC using fused silica capillary column (25 m×0.2 mm) coated with Carbowax 20M at 190 °C with nitrogen carrier gas. Fatty acids were identified by comparison with authentic standards of 16:0, 18:0, 18:1, 18:2(n-6), 18:3(n-3), 18:4, 20:4(n-6) and 20:5(n-3) and by ECL measurement (Christie, 1988).

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