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RESEARCH PAPERS

Fatty Acid Composition of Lipids from Leaves and Strobila of Cycas Revoluta (*Cycas revoluta*)

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Abstract—Fatty acid (FA) composition of lipids from leaves and differentiated fleshy strobila tissues and sporangia with spores of Cycas (*Cycas revoluta* Thumb.) after their step quantitative extraction from plant material was investigated. Quantitative content and qualitative composition of FAs of extractable and nonextractable leaf lipids were determined. It was established that flesh lipids of sporophylls are characterized by a high saturation level and contain a considerable proportion of saturated FAs with the usual chain length (C_{12}–C_{18}, 53–57%). At the same time, total amount of etherified FAs with a very long chain in lipids not extractable by the method of Zhukov and Vereshchagin exceeds several times that found in extractable lipids (~15 and ~4%, respectively). Neutral lipids of Cycas spores were represented by triacylglycerols, the lower-alkyl esters of FAs, free FAs, and sterol esters.

Keywords: Cycas revoluta, fatty acids, microsporangies, microstrobila, sporophylls, sterol esters

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INTRODUCTION

It is known that fatty acid composition of lipids from different parts of gymnosperms (Pinophyta) has been studied many times [1]. However, for such plants as members of the Cycad family (Cycadaceae), fatty acid composition of their lipids before the onset of our work was investigated only for their leaves [1], seeds [2] and pollen (spores) [3]. Plants of this family, together with *Ginkgo biloba*, are the oldest on our planet, and it is thought that they represent the result of symbiosis of plant-host, fungus, two bacterial species, and cyanobacteria formed many years ago [4]. By their origin, Cycadaceae are tightly associated with ferns (section Polypodiophyta), because they are similar to them by a number of signs [4]. Cycads are widely used not only as decorative material but also as a food source. The bark and heartwood of the trunk contains up to 45% starch, which is used for preparation of a specific product, sago. Pinnate cycad leaves are tight and hard with thick layer of cuticle helping the plant to economize not only moisture but also to defend the leaf against penetration into it of pathogenic funguses and bacteria. Cycad leaves possess a whole number of structural peculiarities allowing these plants to live in a dry climate [4].

One of typical representatives of the Cycadaceae family is drooping cycad (*Cycas revoluta* Thunb.), a dioecious plant whose males, like others of Cycadaceae, form microstrobila at the top of the trunk. *C. revoluta* originates from subtropic South Japan.

Because *C. revoluta* flowers extremely rarely (greenhouse specimens can flowering only several times during their growing [4]), lipids of its generative organs were not previously studied. Take into account for this fact and also for the circumstance that one specimen of *C. revoluta* growing in the greenhouse of the Institute of Plant Physiology of the Russian Academy of Sciences under short-light day already for several decades suddenly, for the first time, opened its "flower," it was of indubitable interest to reveal possible peculiarities of lipid composition of some organs of this plant. In the present work, we investigated FA composition of lipids from leaves, tissues of squamiform sporophylls (microstrobila) and microsporangies with spores collected during the flowering of the *C. revolute* male.

MATERIALS AND METHODS

In the experiments performed in the present work, leaves, sporophylls (microstobila), pollen (spores), and microsporangies together with the spores contained in them (pollen) of flowering male plant *Cycas revoluta* Thunb. growing in the greenhouse of the Institute of Plant Physiology of the Russian Academy of Sciences were used.

For the analysis, we used 3–4-year-old leaf segments (16 samples, 8.93 g) without rachises, sporo-

Abbreviations: FA–fatty acid; FALAE–the lower-alkyl esters of fatty acids; FAME–methyl esters of fatty acids; FFA–free fatty acid; SE–sterol ester; TAG–triacylglycerol; VLCFA–very long chain fatty acid; UI–unsaturation index.

phylls (12 samples, 6.05 g), pollen (spores, 1.62 g), and also sporangies collected from sporophylls together with spores contained in them. Before the analysis, sporophylls and leaf segments were treated twice with pure chloroform for a short time (15 sec) by their immersion in it to separate surface lipids.

Plant material was ground in a mortar, and lipids from sporophylls and microsporangies were extracted with chloroform-methanol mixture (2 : 1, 3 × 20 mL), whereas those from leaves were extracted using the method of Zhukov and Vereshchagin [5]. An extract of pollen was fractionate by the method of preparative thin-layer chromatography on Silufol plates in the hexane/ether/acetic acid system (8 : 2 : 0.1, v/v/v). Zones of individual classes of neutral lipids were removed from the plate, transferred onto a glass filter, lipids were eluted from the adsorbent with chloroform, and solvent was evaporated.

Acyl-containing lipids of both extracts and residues of plant material after extraction were subjected to alkaline hydrolysis in 4% ethanol solution of KOH, unsaponifiable lipids were separated with hexane; thereafter, pH of the resulting solution was adjusted to weakly acid reaction and FAs were converted into methyl esters (FAMEs). Qualitative and quantitative composition of individual FAs were determined by the method of gas-liquid chromatography–mass spectrometry (GLC-MS) [6] on an Agilent 7890A GC device (Agilent Thechnologies, United States) equipped with a 60-m capillary column with an inner diameter of 0.25 mm (DB-23, Ser. no. US8897617H). The column contained a grafted (50% cyanopropyl)-methylpolysiloxane polar liquid phase as a 0.25 μm-thick film. Conditions of separation of FAMEs: pressure of carrier gas (helium) in the injector was 191 kPa, work pressure of gas in the column was 245 kPa, flow rate of gas was 1 mL/min, linear rate of carrier gas in the column was 18 cm/sec, volume of sample solution was 1 μL (10 μg FAMEs), flow split ratio was 1 : 5, temperature of evaporator was 260°C. Program of temperature gradient of the column: from 130°C to 170°C with the rate of 6.5°C/min, from 170°C to 215°C with the rate of 2.75°C/min, hold at 215°C for 25 min, from 215°C to 240°C with the rate of 40°C/min, and hold at 240°C for 50 min. The work temperature of MS-detector (5975C MSD) was 240°C. Identification of individual species of FAMEs and calculation of their quantitative content in the mixture were performed with the use of advanced software package MSD ChemStation G1701EA E.02.00.493 with the library of spectra NIST.

Mass of esterified FAs in extract and unextractable residue was established with GLC-MS with the used of the method of the inner standard (margaric acid, 17:0) as described earlier [6, 7]. Since, in the course of preliminary experiments in the extracts obtained, some amount of 17:0 FA of natural origin was found (from 0.3 to 1.3% of total FAs content), it further was taken into account during determination of absolute content of esterified FAs. To this end, FA composition of all samples was determined twice, with the addition of the standard and in the absence of it. To determine structure of unusual C_{15}-FA and position of double bonds in molecules of unsaturated FAs, total FAs were converted into their 4'4'-dimethyl-2-oxazoline derivatives (DMOX) [8] and their composition was analyzed by the method of GLC-MS [6].

Composition of the zone of the lower-alkyl esters of FAs (FALAE) coincided by their chromatographic mobility at TLC with the zone of FAMEs was analyzed by the method of GLC-MS as well [6]. Unsaturation index (UI) of FAMEs was calculated according to formula $UI = \Sigma P_i e_i/100$, where P_i is content of i-th FA species (%) and e_i is a number of double bonds in i-th FA.

In the tables, the means of the data obtained from three independent experiments and their standard deviations are shown.

RESULTS AND DISCUSSION

Composition of esterified FAs of lipids of sporophylls and microsporangies of *C. revoluta* is shown in Table 1. It can be seen that the most diversity appeared to be inherent in lipids of sporophylls (25 individual FA species) unextractable with $CHCl_3 + CH_3OH$ (2 : 1) mixture, whereas sporophyll extractable lipids and total lipids of microsporangies included 20 and 19 species of FAs, respectively. Both extractable and unextractable lipids of sporophylls were enriched with saturated (60.7 and 65.3% of total FAs) and diunsaturated (27.3 and 22.6%) FAs, while lipids of microsporangies, along with these FAs (55.9 and 23.3%), include a appreciable amount of monounsaturated FAs (18.3%). The major FAs in both lipid fractions from sporophyll flesh were represented by palmitic (16:0), stearic (18:0), and linoleic (Δ9,12-18:2) acids, whereas the major acid appeared to be α-linolenic acid (Δ9,12,15-18:3) in extractable lipids. In lipids of microsporangies, predominant FAs were represented by oleic acid (Δ9-18:1) along with 16:0, 18:0 and Δ9,12-18:2.

It shold be noted that both sporophyll lipid fractions studied and microsporangies lipids contained a marked amount of FAs with a very long chain (VLCFAs), which cannot be a components of surface lipids', because the latter were removed before the extraction. Nonextractable sporophyll lipids were characterized by not only the largest relative content (~15% of total FAs) but also by diversity of VLCFAs (nine saturated and unsaturated FA species). A somewhat less content of such FAs (12.9%) was found in lipids of microsporangies, which were represented by only saturated $C_{20\text{-}24}$ FAs. In all cases, the major FA of such type was behenic acid (22:0, 1.6–6.6%).

As far as we know, up to the onset of our investigation, lipids of sporophylls and microsporangies of

Table 1. Composition of etherified FA of lipids of *Cycas revoluta* sporophylls and microsporangies, % of total summa of MEFAs

Fatty acids	Sporophylls		Microsporangies
	extractable lipids	unextractable lipids[1]	total lipids[2]
12:0	3.8 ± 0.1	1.3 ± 1.1	0.2 ± 0.1
14:0	4.6 ± 0.1	2.0 ± 0.2	0.8 ± 0.2
15:0	0.2 ± 0.1	1.5 ± 0.0	0.6 ± 0.1
16:0	42.8 ± 0.4	40.2 ± 1.4	35.5 ± 0.5
Δ7-16:1	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.0
Δ9-16:1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Δ7,10,13-16:3	0.2 ± 0.0	0.3 ± 0.2	–
17:0	1.2 ± 0.0	1.0 ± 0.0	0.6 ± 0.0
18:0	4.4 ± 0.1	6.3 ± 0.1	5.1 ± 0.1
Δ9-18:1	3.8 ± 0.1	4.4 ± 0.1	16.4 ± 0.1
Δ11-18:1	1.2 ± 0.0	1.0 ± 0.0	0.9 ± 0.0
Δ9,12-18:2	27.0 ± 0.3	22.2 ± 0.3	23.0 ± 0.2
Δ9,12,15-18:3	6.1 ± 0.6	3.9 ± 0.1	2.5 ± 0.0
19:0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
20:0	0.9 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Δ11,14-20:2	0.3 ± 0.0	0.4 ± 0.0	–
21:0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
22:0	1.6 ± 0.1	5.9 ± 0.3	6.6 ± 0.3
23:0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
24:0	0.7 ± 0.0	3.0 ± 0.3	3.6 ± 0.2
Σ VLCFAs	3.9 ± 0.1	14.9 ± 0.1	12.9 ± 0.1
UI	0.792 ± 0.016	0.687 ± 0.004	0.724 ± 0.003

[1] 14-Me-15:0 (0.2 ± 0.0%), Δ11-20:1 (0.4 ± 0.0%), Δ5,11,14-20:3 (1.5:0.0%) and 26:0 (1.1 ± 0.5%) FAs were present as well.
[2] Δ5,9-18:2 (0.3 ± 0.0%) FAs—was present as well.

Cycadaceae family members were not studied. Earlier, we investigated FA composition of total lipids from *C. revoluta* pollen [3]. These exhibited somewhat more various composition of FAs (28 individual species) than lipids of sporophylls and microsporangies, largely thanks to a large number of Δ5-FAs. It appeared that sporophylls and microsporangies differ from pollen by a higher level of 16:0 FA and lower concentration of Δ9-18:1 and Δ9,12-18:2 FAs.

In the neutral fraction of extractable lipids from *C. revoluta* pollen, we identified triacylglycerols (TAGs), FALAE, free FAs (FFAs), and sterol esters (SE), which FA composition is shown in Table 2; composition of polar lipids of *C. revoluta* pollen was not investigated. In lipid classes of *C. revoluta* pollen studied, 28 individual species of C_{12}-C_{28}-FAs were identified, including three saturated FAs with branched chain, and fractions of TAGs and FFAs exhibited the most diversity (22 species for each FA). As in total pollen lipids [3], in TAGs, FFAs, and SE, palmitic, stearic, oleic, and linoleic acids were the predominant ones, and margaric acid (17:0) was one of the major FA only in FALAE fraction. FALAE of Cycad pollen were represented mostly by FAMEs (91.4% of total FALAE) and also contained isopropyl and ethyl esters of FAs (5.2 and 3.1%, respectively), with isopropyl myristate and ethyl palmitate the predominant ones among the latter.

Earlier mixtures of FALAE that were products of cell biosynthesis rather than experimental artifacts were isolated in a small amount from chloroform extracts of arils and seeds of four species of euonymus (*Euonymus* sp.) fruits, which consisted of methyl, ethyl, *n*-propyl, and *n*-butyl esters of saturated, mono-, di- and triunsaturated C_{14}-C_{18}-FAs [6]. It should be noted that FALAE are relatively rare in the occurrence in plants; they were found in gymnosperms (fir, spruce) and liverwort *Conocephalum conicum* along with angiosperms. However, unlike cycad pollen where FALAE contain exclusively methyl, ethyl and isopropyl ethers of $C_{\leq 18}$-FAs, they are represented in needles of fir and spruce by only methyl esters of C_{22}-C_{34}-FAs [9], whereas by ethyl esters of C_{12}-C_{24}-FAs in liverwort [10].

Table 2. Composition of esterified FAs of individual classes of *Cycas revoluta* spores lipids extractable by $CHCl_3$

Fatty acids	TAG	FALAE[1]	FFAs[2]	SE
12:0	–	3.7 ± 2.6	0.8 ± 0.0	–
anteiso-15:0	0.4 ± 0.1		0.4 ± 0.0	
14:0	3.4 ± 0.2	4.8 ± 2.7	3.4 ± 0.1	–
15:0	0.9 ± 0.1	2.1 ± 1.2	1.0 ± 0.0	–
16:0	25.7 ± 0.6	23.2 ± 1.1	46.2 ± 0.5	36.1 ± 0.2
Δ7-16:1	1.9 ± 0.1	0.4 ± 0.0	1.0 ± 0.1	–
Δ9-16:1	0.3 ± 0.2	0.2 ± 0.0	–	–
anteiso-17:0	0.1 ± 0.1	–	–	–
17:0	2.2 ± 0.0	23.0 ± 1.7	2.4 ± 0.0	–
iso-18:0	0.2 ± 0.1	1.0 ± 0.0	–	–
18:0	7.7 ± 0.0	7.5 ± 0.1	6.6 ± 0.0	16.6 ± 0.9
Δ7-18:1	0.2 ± 0.2	–	–	–
Δ9-18:1	17.0 ± 0.3	10.9 ± 0.3	6.3 ± 0.0	15.4 ± 0.2
Δ11-18:1	0.8 ± 0.0	0.5 ± 0.1	1.2 ± 0.1	–
Δ9,10-18:2	0.7 ± 0.1	–	–	–
Δ9,12-18:2	19.9 ± 0.1	12.8 ± 3.6	16.7 ± 0.1	31.9 ± 1.1
Δ9,12,15-18:3	0.9 ± 0.1	1.1 ± 0.1	2.3 ± 0.4	–
19:0	–	–	0.1 ± 0.0	–
20:0	0.3 ± 0.3	–	1.0 ± 0.0	–
Δ11-20:1	–	–	0.4 ± 0.0	–
Δ8,11-20:2	–	–	0.2 ± 0.0	–
Δ11,14-20:2	–	–	0.1 ± 0.0	–
Δ5,11,14-20:3	–	–	1.1 ± 0.0	–
22:0	0.3 ± 0.0	–	4.7 ± 0.2	–
23:0	0.4 ± 0.3	–	0.1 ± 0.1	–
24:0	10.7 ± 0.2	1.3 ± 0.9	3.5 ± 0.2	–
26:0	3.6 ± 0.1	–	0.5 ± 0.1	–
28:0	1.5 ± 0.0	–	–	–
Σ VLCFAs	16.5 ± 0.2	1.3 ± 0.9	10.6 ± 0.1	–
UI	0.600 ± 0.021	0.409 ± 0.069	0.533 ± 0.011	0.792 ± 0.035

[1] % of total FALAE.
[2] Isopropyl esters of miristic (4.6 ± 0.8%) and *anteiso*-17:0 (14-methyl-16:0) (0.7 ± 0.4%) FAs and also ethyl esters of palmitic (1.5% ± 0.7%) and margaric (0.7 ± 0.5%) acids were present as well.

In Table 3, the data of absolute and relative content of FAs of *C. revoluta* leaf lipids being extracted as well as unextractable ones and FA composition of surface leaf lipids are shown. It can be seen that absolute lipid content in *C. revoluta* leaves amounted to about 1% per their dry mass without taking into account surface lipids while an extent of their extraction was 93.3%. Surface lipids (15 individual species) exhibited the lowest diversity, and saturated and monounsaturated FAs (53.1 and 38.8% of total FAs, respectively) were predominant among them; in this fraction, major FAs were represented by 16:0, Δ9-18:1 and 18:0 ones. At the same time both extractable and unextractable lipids of *C. revoluta* leaves were distinguished for considerably higher diversity of FAs (26 and 22 species, respectively) and, along with usual *n*-C_{12-19} FAs, included approximately 2–3% of C_{15}- and C_{18}-FAs containing double bond in *cis*-Δ5 position (Δ5-FA), 2.3–4.6% of VLCFAs, trace amounts of *anteiso*-13:0 and *anteiso*-15:0 FAs, and also a small amount of Δ9,12-17:2 and Δ9,11-18:2 FAs not contained in surface lipids. In extractable lipids saturated, di- and triunsaturated FAs (34.2, 31.0, and 25.1% of total FAs, respectively) were dominant ones, while such FAs were represented by saturated, mono- and diunsaturated FAs in unextractable lipids (50.5, 17.6, and 21.7% of total FAs, respectively). Major FAs of extractable leaf

Table 3. Content and composition of etherified FAs in lipids of *Cycas revoluta* leaves

Fatty acids	Surface lipids[1], mass %	Extract of leaf lipids[2]			Residue after extraction		
		mass %	μg/g fresh mass	μg/g dry mass	mass %	μg/g fresh mass	μg/g dry mass
12:0	5.1 ± 0.6	0.6 ± 0.0	23 ± 1	53 ± 2	2.9 ± 0.2	5 ± 0	11 ± 1
13:0	0.3 ± 0.3	–	–	–	0.9 ± 0.3	1 ± 1	3 ± 1
14:0	3.7 ± 0.1	2.7 ± 0.1	107 ± 3	252 ± 6	2.7 ± 0.1	107 ± 3	252 ± 6
12-Me-14:0	0.1 ± 0.1	–	–	–	0.3 ± 0.0	1 ± 0	1 ± 0
15:0	1.7 ± 0.8	0.4 ± 0.0	15 ± 0	36 ± 0	1.8 ± 0.0	3 ± 0	7 ± 0
Δ5-15:1	1.3 ± 0.0	1.3 ± 0.0	50 ± 1	118 ± 2	0.2 ± 0.2	0 ± 0	1 ± 1
16:0	30.5 ± 0.9	22.9 ± 0.0	910 ± 5	2135 ± 11	27.6 ± 0.5	46 ± 1	109 ± 1
Δ7-16-1	1.3 ± 0.2	0.2 ± 0.0	7 ± 0	17 ± 0	1.1 ± 0.0	2 ± 0	4 ± 0
Δ9-16:1	0.2 ± 0.2	1.4 ± 0.0	56 ± 0	131 ± 1	0.8 ± 0.0	1 ± 0	3 ± 0
Δ7, 10-16:2	–	1.7 ± 0.1	68 ± 1	159 ± 3	0.5 ± 0.0	1 ± 0	2 ± 0
17:0	1.2 ± 0.0	1.0 ± 0.0	37 ± 1	87 ± 2	1.0 ± 0.0	2 ± 0	4± 0
18:0	10.5 ± 2.1	3.8 ± 0.1	150 ± 4	353 ± 10	8.7 ± 0.1	15 ± 0	34 ± 0
Δ9-18:1	33.7 ± 1.5	5.7 ± 0.3	225 ± 13	528 ± 31	14.3 ± 0.7	24 ± 1	57 ± 2
Δ11-18:1	2.0 ± 0.1	1.1 ± 0.0	45 ± 2	105 ± 4	1.2 ± 0.0	2 ± 0	5 ± 0
Δ5,9-18:2	–	0.4 ± 0.3	15 ± 10	35 ± 23	0.4 ± 0.3	15 ± 10	35 ± 23
Δ5,12-18:2	–	1.5 ± 0	60 ± 2	141 ± 4	1.7 ± 0.4	3 ± 1	7 ± 2
Δ9,12-18:2	8.1 ± 0.1	27.1 ± 0.1	1073 ± 19	2520 ± 45	19.1 ± 0.2	32 ± 0	75 ± 0
Δ9,12,15-18:3	–	23.9 ± 0.1	946 ± 15	2221 ± 36	10.2 ± 0.4	17 ± 1	41 ± 2
19:0	–	0.5 ± 0	18 ± 1	43 ± 3	–	–	–
20:0	–	1.1 ± 0.1	42 ± 5	99 ± 12	3.1 ± 0.8	5 ± 1	12 ± 3
21:0	–	0.1 ± 0.0	4 ± 2	9 ± 4	–	–	–
22:0	–	0.8 ± 0.0	32 ± 1	75 ± 3	0.5 ± 0.1	1 ± 0	2 ± 1
23:0	–	–	–	–	0.2 ± 0.1	0.4 ± 0	1 ± 0
24:0	–	0.3 ± 0.0	13 ± 1	30 ± 2	0.8 ± 0.3	1 ± 1	3 ± 1
Summa	100	100	3960 ± 54	9298 ± 128	100	284 ± 1	668 ± 2
UI	0.550 ± 0.040		1.470 ± 0.023			0.916 ± 0.041	

[1] Δ11-16:1 (0.3 ± 0.3%) FAs were present as well.
[2] 10-Me-12:0 (trace amount), Δ7,10,13-16:3 (1.3 ± 0.1%), Δ9,12-17:2 (0.2 ± 0.0%) and Δ9,11-18:2 (0.1 ± 0.0%) FAs were present as well.

lipids were 16:0, Δ9-18:1, 9,12-18:2, and Δ9,12,15-18:3, while unextractable lipids contained the same major FAs and 18:0 as well. Unextractable lipids (6.7% of total esterified FAs) exceeded extractable ones by relative content of saturated FAs (by ~30%), mainly 16:0 and 18:0, and monounsaturated FAs (by 45%), but they are second to them by level of Δ9,12-18:2 and Δ9,12,15-18:3 (by 42% and 2.3 times, respectively) that explains their considerably lower (by ~38%) value of UI.

As it was already noted above, earlier FA composition of lipids was studied in leaves [1], seeds [2], and pollen [3] of *C. revoluta*. Analysis of the data obtained in these studies showed that, in all cases, composition of FAs of their lipids exhibited large diversity (>20 individual species of FAs) and was distinguished for a considerable content of VLCFAs (14.9, 6.9, and 5.0% of summa of FAs) and also $C_{18\text{-}20}$ FAs containing a double bond in the Δ5-position (7.8, 4.2, and 4.9%, respectively). Here, it should be noted that the presence of Δ5-FA in lipids is thought to be a distinctive feature of gymnosperm [1]. Thus, Mongrand et al. [1] earlier investigated FA composition of leaf lipids of 137 species of Gymnospermae belonging to 14 families, including *C. revoluta* leaves. According to the data of this work, lipids from these plants contained a small amount of *anteiso*-17:0 FA, taxoleic (Δ5,9-18:2), pinolenic (Δ5,9,12-18:3), coniferonic (Δ5,9,12,15-18:4), Δ5,11-20:2, sciadonic (Δ5,11, 14-20:3) and juniperonic (Δ5,11,14,17-20:4) acids and also a nonidentified FA whose methyl ester was eluted from the gas chromatographic column directly before methyl palmitate [1]. Lipids of two other studied species of *Cycas* exhibited similar FA composition [1]. At the same time, as follows from Table 3, in lipids of *C. revoluta* leaves, we did not find the above FAs, but we identified *anteiso*-13:0, *anteiso*-15:0, *anteiso*-17:0, Δ5,12-18:2 FAs,

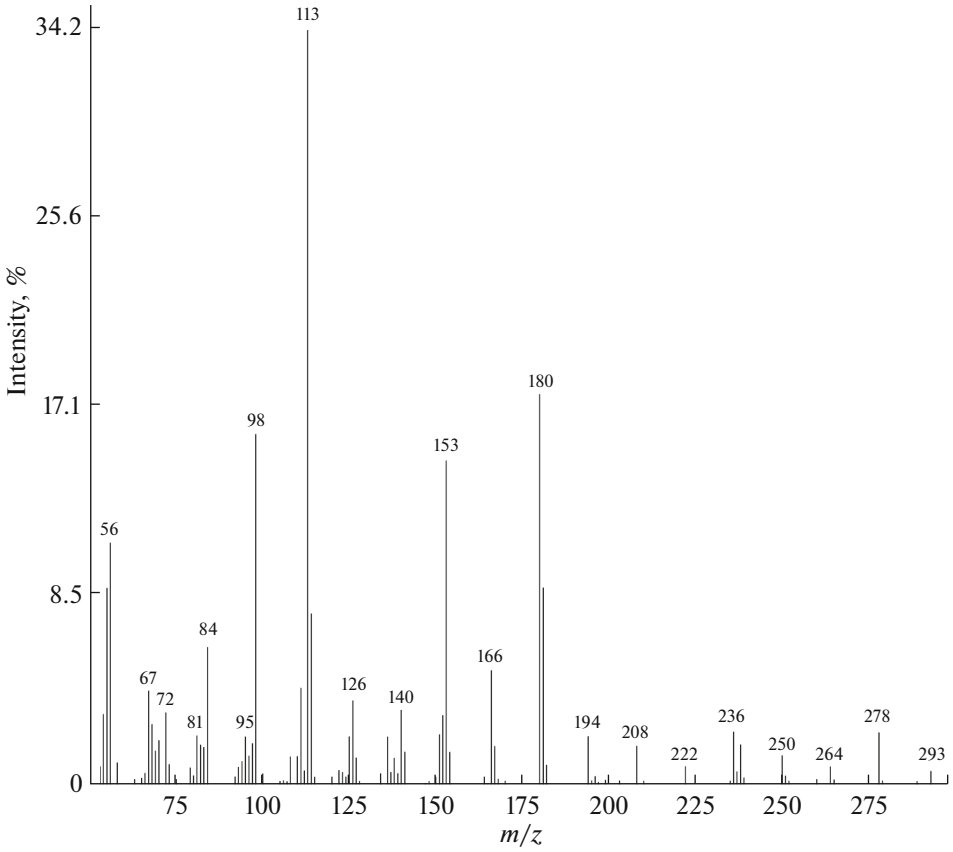

Fig. 1. Mass-spectrum of DMOX-derivative of Δ5-pentadecenic acid from lipids of *C. revoluta* leaves. Intensities of fragmentary ions are shown relative to that of an ion with $m/z = 113$, one of the ions characteristic of DMOX-derivative of this FA is taken as 100%.

and also Δ5-15:1 FA, whose mass-spectrum of DMOX derivative is shown in the Fig. 1. It can be seen that this FA represents 15:1 FA with an unusual position of a double bond, M^+ at *m/z* 293, while the presence of ion with *m/z* 153 indicates the location of a double bond, namely in the Δ5-position of the molecule [8]. Earlier, FA with analogues retention parameters at GLC was found by Mongrand et al. in lipids of *C. revoluta* leaves; these authors, however, could not identify it [1].

As far as by their origin, *Cycas* is tightly associated with ferns, it was of interest to compare FA composition of lipids of pollen (spores) and *C. revoluta* leaves with analogous data obtained for ferns. According to Lytle et al. [11], in lipids of spores from five fern species, saturated *n*-C_{10-30} FAs were found, including ten individual species of FAs with odd number of carbon atoms and also Δ9-18:1, Δ9,12-18:2, and Δ9,12,15-18:3 FAs. All the species of ferns studied contained a considerable amount of VLCFAs (12.4–32.2% of total FAs). In these species, dominant FAs in spore lipids were saturated ones (46.3–93.3%), and major FA was the 16:0 (23.0–52.6%). In some fern species studied by Lytle et al., Δ9-18:1, Δ9,12-18:2, 22:0, 24:0, and 30:0 appeared to be major FAs [11]. However, the results of analysis performed by Gemmrich who determined FA composition of lipids from 16 fern species, including some ferns studied by Lytle et al., and no marked amount of VLCFAs or Δ5-FAs was found, while major FAs were represented by 16:0, Δ9-18:1, and Δ9,12-18:2 [12]. In lipids of pinnae leaves of other fern species studied by Jamieson and Reid [13], 25 species of C_{12-22} saturated, mono-, di-, tri-, tetra- and pentaunsaturated FAs were identified, and the major among them appeared to be 16:0, Δ7,10,13-16:3, Δ9,12-18:2, Δ9,12,15-18:3, and Δ5,8,11,14-20:4 FAs. In these ferns in lipids of their leaves, 11–18.5% of VLCFAs was found, while Δ5-FAs were absent in them according to the results obtained by Jamieson and Reid [13].

As far as Δ5-FAs is concerned, which, as a rule, are present in any amount in lipids of gymnosperms, these acids were either absent in lipids of sporophylls, microsporangies, and Cycas leaves or were represented by only one to three individual FAs species (Table 3), while pollen diversity of Δ5-FAs was considerably higher (six and four individual species of FAs) in lipids of leaves and *C. revoluta* according to Mongard et al. [1] and Sidorov et al. [3]. Although the physiological role of these FAs remains unclear, it is suggested that their presence in gymnosperms is somewhat associated with plant cold tolerance. This

suggestion is supported in part by absence of Δ5-FAs in lipids from leaves of *Welwitschia mirabilis,* tropical pond dweller, and their high content in frost resistant conifers (to 13%, 17.3%, and 22% in fir, pine, and larch, respectively) [1]. Seeds of many gymnosperms exhibit a considerable content of Δ5-FAs as well. For example, *Pinus sylvestris, P. strobus, P. peuce* [14], and *Taxus chinensis* [1] contain 31, 29.7, 29.4, and 23.7% of Δ5-FAs, respectively. These FAs are found in lipids of other organisms as well, for example in lichens [15].

Thus, it can be concluded that lipids of different organs of Cycas are characterized by considerable diversity of FAs composition, including VLCFAs and Δ5-FAs. Photosynthesizing organs (leaves) of *C. revoluta* differ from generative ones (sporophylls, pollen, and microsporangia) by considerably higher unsaturation of FAs (UI = 1.467, 0.792, 1.171, and 0.724, respectively), the presence of Δ5-15:1 FAs, and the absence of FAs with a branched chain.

In addiiton, pollen lipids contain FALAE, which are comparatively rarely found in plants, and, in addition to Cycas pollen, revealed earlier in fruits of some euonymus, needles of a number of gymnosperms and little number of other plant objects.

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