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ORIGINAL PAPER

Positional-Species Composition of Triacylglycerols from the Arils of Mature Euonymus Fruits

Roman A. Sidorov · Vasily P. Pchelkin · Anatoly V. Zhukov · Andrei G. Vereshchagin · Vladimir D. Tsydendambaev

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Abstract Positional-species composition (PSC) of 1,2,3-triacyl-*sn*-glycerols (TAG) from the arils of mature fruits of 13 species of *Euonymus* L. genus was established. The residues of six major fatty acids (FA), palmitic, stearic, hexadecenoic (H), octadecenoic (O), linoleic (L), and linolenic, were present in the TAG. PSC of TAG was determined by their partial lipase hydrolysis. By using hierarchical cluster and principal component analyses, it was definitely demonstrated that separate taxonomic units forming this genus were significantly distinguished as regards PSC of TAG. In particular, the *Euonymus* subgenus greatly exceeded the *Kalonymus* subgenus in both total content of L in TAG and in the rate of its incorporation into their mid-position, while TAG of *Kalonymus* were marked by a prevalence of O-TAG and *sn*-2-O isomers. Thus, these subgenera were significantly distinct in the rate of incorporation of O and L residues in the *sn*-2 position of TAG molecules. Meanwhile, the TAG from the *Euonymus* section species were marked by an enhanced concentration of H and the incorporation of H in **UUU** TAG was much more active than in other TAG types. As for positional-type composition of TAG, saturated FA were always virtually absent in the *sn*-2 position of *Euonymus* aril TAG.

Keywords *Euonymus* species · 1,2,3-Triacyl-*sn*-glycerols · Positional-species composition · Type-species composition · Fatty acids · Taxonomic position

Deceased: A. G. Vereshchagin.

R. A. Sidorov (✉) · V. P. Pchelkin · A. V. Zhukov · A. G. Vereshchagin · V. D. Tsydendambaev
Laboratory of Lipid Metabolism, K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35, Moscow 127276, Russia
e-mail: roman.sidorov@mail.ru; vdt@ippras.ru

Abbreviations

A	Octadecenoic or linoleic acid
DAG	Diacylglycerol
EF	Enrichment factor
FA	Fatty acid
FAME	FA methyl ester
H	Hexadecenoic
L	Linoleic
Ln	Linolenic
O	Octadecenoic
P	Palmitic
PCA	Principal component analysis
PSC	Positional-species composition
PTC	Positional-type composition
RRD	2-Random 1,3-random distribution
S	Total saturated FA
SD	Standard deviation
SF	Selectivity factor
St	Stearic
TAG	1,2,3-Triacyl-*sn*-glycerol
U	Total unsaturated FA
U_2	U content in *sn*-2 position of TAG
U_T	U content in *sn*-1,2,3 positions of TAG

Introduction

The *Euonymus* L. genus from Celastraceae family is represented by shrubs or woody plants and is distributed mostly in many countries of the northern hemisphere including Russia [1, 2]. According to one of the most recent taxonomy systems [3], *Euonymus* L. species are subdivided into two subgenera, *Euonymus* and *Kalonymus,* and a number of sections.

It has long been known that the fruits of these species contain fatty oils [4]. They have been shown to be

accumulated not only in the seeds but also in the fruit arils, and almost all of reserve 1,2,3-triacyl-*sn*-glycerols (TAG) of the oil-bearing fruits are concentrated in their arils [5]. Previously, we have determined the quantitative content and major fatty acid (FA) composition of aril TAG in several species of the *Euonymus* L. genus. It was found that separate taxonomic units of this genus differ from each other in the concentration of major FA. Thus, by using statistical analysis, it was demonstrated that the aril TAG of *Euonymus* subgenus were characterized by an increased content of linoleate (L), while those from the *Kalonymus* subgenus by the predominance of octadecenoate (O) [6]. In this connection, it was of interest to characterize TAG positional-species composition (PSC [7]) in the fruit arils of a number of various taxonomical units of the *Euonymus* L. genus. Moreover, in future studies, it will be worthwhile to compare the PSC of aril TAG with the PSC of the quite unusual 3-acetyl-1,2-diacyl-*sn*-glycerols predominating in the seed oil of the same euonymus fruit [5]. Hence, the determination of PSC of TAG from the arils of mature fruits of several *Euonymus* L. species, which has not been investigated thus far, was the subject of the present investigation.

Materials and Methods

Plant Material

Only small amounts of TAG, scarcely sufficient for PSC analysis, were found in *E. verrucosus* fruit arils [6], and therefore this species which has been studied previously was excluded from the present investigation. Mature fruits of 13 species belonging to two subgenera and four sections of the genus *Euonymus* L. were collected during 2010–2011 in the arboretum of the Main Botanical Garden of RAS (Moscow). Herbarium voucher specimens were deposited in the Herbarium of this Garden. Aril separation from fruits, extraction of total lipids, TLC lipid fractionation, and preparation of a pure TAG sample were all carried out [5, 8].

Determination of Content and FA Composition of TAG

Qualitative and quantitative FA composition in TAG preparations and in *sn*-1,2(2,3)-diacylglycerols (*rac*-DAGs) was determined by GC–MS [5] using an Agilent 7890A GC device (Agilent Technologies, USA) fitted with a capillary column (DB-23, Ser. no. US8897617H, 60 m × 0.25 mm) containing a grafted (50 % cyanopropyl)-methylpolysiloxane polar liquid phase as a 0.25-μm-thick film. The FAME were separated under the following conditions: operational carrier gas (helium) flow in the column, 1 mL/min; sample volume, 1 μL (~10 μg FAME); flow split ratio, 1:20; and evaporator temperature, 260 °C. The oven temperature program was as follows: from 130 to 170 °C at 6.5 °C/min, to 215 °C at 2.75 °C/min (25 min at this temperature), to 240 °C at 40 °C/min, and 50 min at 240 °C, operational temperature of the mass selective detector (5975C MSD), 240 °C, ionisation energy, 70 eV. For identifying individual FAME species and calculating their concentrations in the mixture, a NIST search library and the MSD Chem Station software were used [5].

Determination of TAG Positional-Species Composition

PSC of TAG was determined by their partial lipase hydrolysis and subsequent analysis of FA composition of *sn*-1,2(2,3)-diacylglycerols (*rac*-DAGs) as products of this reaction [9]. Initial TAG (25 mg) mixed with 12 ml of 0.25 M aqueous tris (hydroxymethyl) aminomethane buffer solution (pH 7.5; Sigma-Aldrich, USA), 250 μL of 0.01 M $CaCl_2$ monohydrate, and 625 μL of 0.1 % deoxycholic acid sodium salt monohydrate solution (D6750; Sigma, USA) were kept for 5 min at 37 °C. After adding 38 mg of lipase from porcine pancreas (L3126; Sigma), the mixture was stirred and incubated for 30 min at 37 °C. To stop the reaction, 6 M HCl (1.25 ml) and freshly distilled EtOH (3 ml) were added, and the lipids thrice extracted with benzene, which was then distilled-off using a rotary evaporator. The lipid residue was quantitatively transferred on a TLC plate starting zone using *n*-hexane and a microsyringe. The TLC plate (Sorbfil, Russia) was preliminarily washed with an acetone-MeOH (1:1) mixture and impregnated with a 6 % methanolic solution of H_3BO_3 containing 0.01 % 2′,7′-dichlorofluorescein (ACROS Organics, USA). The plate was exposed in a TLC chamber containing EtOH-stabilized (3 % v/v) $CHCl_3$:acetone (19:1 v/v) as a mobile phase. Immediately after the phase front reached the top edge of the plate, it was removed from the chamber and kept in a hood at room temperature for 5 min. The position of the *rac*-DAG zone ($R_f \approx 0.7$) was marked with a needle under UV ($\lambda = 254$ nm). The sorbent was transferred onto a glass filter No.3, and *rac*-DAGs were eluted by a $CHCl_3$:MeOH mixture (96:4 v/v). Then, the *rac*-DAGs (1.5–1.8 mg) were converted into FA methyl esters (FAME), and their composition was determined by GC–MS [5].

Equations described earlier [10] were employed for calculating FA composition of *sn*-1,2,3, *sn*-2, and *sn*-1,3 positions, as well as the PSC of TAG; positional-type composition (PTC) of TAG was estimated using the PSC data [11]. For computing the PSC of TAGs, which include various individual FA residues (a, b, c, etc.; see Table 1), which form the aaa, aab, aba, and abc types of TAGs, the following equations were used [7]:

$$[aaa] = [a]_{1,3}^2 \times [a]_2 \times 10^{-4}$$

Table 1 FA composition of *sn*-1,2,3, *sn*-2, and *sn*-1,3 positions of TAG from mature fruit arils of *Euonymus* L. species in mol %

Euonymus L. species	FA positions of TAG	Fatty acids (% of total FA)					
		P	St	H	O	L	Ln
Subgenus *Euonymus*, Section *Euonymus*							
E. bungeanus	*sn*-1,2,3	28.1	1.5	10.2	17.2	41.7	1.3
	sn-2	0	0	0.2	11.2	86.4	2.1
	sn-1,3	40.0	2.4	14.0	21.0	21.5	1.0
E. europaeus	*sn*-1,2,3	23.2	1.5	6.8	20.5	45.6	2.5
	sn-2	0	0	2.7	12.5	81.1	3.7
	sn-1,3	28.6	2.8	6.9	26.8	32.8	2.1
E. hamiltonianus	*sn*-1,2,3	33.6	2.5	2.0	16.4	44.0	1.5
	sn-2	0	0.3	2.1	15.0	79.8	2.8
	sn-1,3	47.3	3.7	1.7	17.9	28.4	1.0
E. phellomanus	*sn*-1,2,3	31.9	5.3	1.3	18.3	41.3	1.8
	sn-2	0.4	0	0	10.8	86.6	2.1
	sn-1,3	44.7	8.8	1.9	22.6	20.5	1.5
E. semiexsertus	*sn*-1,2,3	28.8	1.6	0.6	16.6	51.3	1.2
	sn-2	0.1	0	0.2	7.3	90.5	1.9
	sn-1,3	40.2	2.5	0.9	21.8	33.9	0.8
E. sieboldianus	*sn*-1,2,3	20.0	2.1	13.6	25.5	37.8	1.0
	sn-2	0.1	0	7.0	15.6	75.4	2.0
	sn-1,3	28.2	3.3	15.6	31.5	20.8	0.6
Subgenus *Euonymus*, Section *Melanocarya*							
E. alatus	*sn*-1,2,3	24.6	2.2	0.5	23.5	46.4	2.8
	sn-2	0.5	0.1	0.4	18.4	76.7	3.9
	sn-1,3	34.1	3.3	0.4	26.9	33.0	2.3
E. sacrosantcus	*sn*-1,2,3	22.3	2.0	0.1	28.1	44.6	2.9
	sn-2	0.5	0.3	0.1	16.8	77.5	4.9
	sn-1,3	30.4	2.8	1.4	34.3	29.1	2.0
Subgenus *Euonymus*, Section *Pseudovyenomus*							
E. pauciflorus	*sn*-1,2,3	26.7	5.4	1.0	39.4	26.5	1.1
	sn-2	0.5	0	0	43.6	54.0	1.9
	sn-1,3	36.8	8.7	1.3	38.7	13.8	0.7
Subgenus *Kalonymus*							
E. latifolius	*sn*-1,2,3	19.1	2.0	1.4	56.5	18.4	2.6
	sn-2	0.2	0	1.3	63.2	30.7	4.6
	sn-1,3	27.1	3.1	1.4	54.2	12.6	1.7
E. macropterus	*sn*-1,2,3	44.2	2.7	0.6	19.8	30.2	2.6
	sn-2	0.2	0.7	0.8	33.0	60.0	5.4
	sn-1,3	62.0	3.8	0.5	14.8	17.6	1.4
E. maximowiczianus	*sn*-1,2,3	34.4	3.0	0.7	29.9	30.9	1.0
	sn-2	0	0	0.1	40.3	57.8	1.7
	sn-1,3	49.5	5.0	1.0	25.6	18.2	0.7
E. sachalinensis	*sn*-1,2,3	33.7	5.3	0.4	47.2	11.9	0.7
	sn-2	0.4	0	0	68.2	30.8	0.6
	sn-1,3	47.1	9.6	0.6	39.2	2.9	0.6

$$[aab] = 2[a]_{1,3} \times [a]_2 \times [b]_{1,3} \times 10^{-4};$$

$$[aba] = [a]^2_{1,3} \times [b]_2 \times 10^{-4}; \text{ and}$$

$$[abc] = 2[a]_{1,3} \times [b]_2 \times [c]_{1,3} \times 10^{-4}.$$

It can be seen that, to perform this calculation, not only the $[a]_{1,2,3}$ and $[a]_{1,2}$ data but also the values of $[a]_{1,3}$ were

Table 2 Enrichment factors (EF) and selectivity factors (SF) of incorporation of major unsaturated FA (O and L) into the *sn*-2 position of TAG of mature fruit arils of *Euonymus* L. species

Euonymus L. species	EF_O	EF_L	EF_L/EF_O	SF_O	SF_L
Section *Euonymus*					
E. bungeanus	0.65	2.07	3.20	0.46	1.46
E. europaeus	0.61	1.78	2.90	0.46	1.34
E. hamiltonianus	0.91	1.81	2.00	0.58	1.16
E. phellomanus	0.59	2.10	3.60	0.37	1.32
E. semiexsertus	0.44	1.76	4.00	0.31	1.22
E. sieboldianus	0.61	1.99	3.30	0.48	1.55
Section *Melanocarya*					
E. alatus	0.78	1.65	2.20	0.57	1.21
E. sacrosanctus	0.60	1.74	2.90	0.46	1.33
Section *Pseudovyenomus*					
E. pauciflorus	1.11	2.04	1.80	0.76	1.39
Section *Kalonymus*					
E. latifolius	1.12	1.67	1.50	0.89	1.33
E. macropterus	1.67	1.99	1.20	0.89	1.06
E. maximowiczianus	1.35	1.87	1.40	0.84	1.17
E. sachalinensis	1.45	2.59	1.8	0.89	1.59

necessary. These values were computed [11] according to the equation:

$$[a]_{1,3} = \left(3[a]_{1,2,3} - [a]_2\right) \times 2^{-1}.$$

To demonstrate the extent of affinity of O and L acids to the *sn*-2 position of TAG, the "enrichment factor", $EF = [A]_2{:}[A]_T$ was used (A = O or L), and the "selectivity factor", $SF = EF{:}([U]_2{:}[U]_T)$ was employed for a comparative estimation of such affinity in the TAG of different levels of unsaturation, T designating total TAG, and 2 the *sn*-2 position of TAG [11]. The EF is the ratio of the molar concentration of an acyl group in the *sn*-2 position to its concentration in the total TAG. The SF is an EF of a particular FA divided by the EF for all FA which are preferentially esterified at the *sn*-2 position [12]. The EF can have any value between 0 and 3; values <1.0 indicate a preference for positions *sn*-1 and *sn*-3, and values >1.0 for position *sn*-2 [7, 12]. The concept of an EF is useful when comparing values for FA competing for the *sn*-2 position in the same oil, but it is less convenient for discussing the behavior of FA in several different oils, and for this reason Gunstone et al. [12] proposed the SF which compensates for some existing difficulties and makes comparison between oils easier and more meaningful.

Statistical Analysis

All experiments were performed in triplicate. Tables and figures show the means of *P* weight values; in all cases, SD did not exceed 10 % of a mean value. Normality of distribution of experimental values in samples was tested using a Shapiro–Wilk criterion. All numerical values obtained here, concentrations of FA and TAG, mol %, and relative units, were characterized by normal distribution ($0.79 \leq W < 1.0$, $0.26 < p < 0.56$). Significance of differences between means in the samples was tested by dispersion analysis (one-way analysis of variation, ANOVA). The significance of differences between various taxonomical units inside the *Euonymus* L. genus as regards the composition of their fruit aril TAG was established by hierarchical cluster analysis—Ward's minimum variance method—and also by clustering using a *k*-means concept; principal component analysis (PCA) was also used. All statistical analyses were carried out with the software Statistica v.10 programs (StatSoft, USA).

Results and Discussion

Positional-Species Composition of TAG

FA composition of intact TAG and their mid- (*sn*-2) and extreme (*sn*-1,3) positions is shown in Table 1. Its data were used for calculating the rate of incorporation of major unsaturated FA (O and L) into the mid-position of TAG (Table 2), as well as the PSC of TAG (Tables 3, 4). Recently, when performing a similar study on TAG from sunflower oils but also using a GC separation of TAG, it was established that the actual GC profiles of TAG matched closely the theoretical calculations made from lipolysis data, thus reinforcing the applicability of such calculations for the identification of the components of GC peaks in oils, complying with the theory of 2-Random 1,3-Random Distribution (RRD) [13]. It follows that PSC of TAG calculated here (Tables 3, 4) must also be considered as being a reliable one.

As shown in Table 1, the mid-position was always almost totally devoid of saturated FA (**S** = St + P), as it is also the case in nearly all TAG of plant origin [7]. Thus, even in the water-rich arils, the TAG were nevertheless synthesized in conformity with the same theory of RRD as in the seeds, which dry in the course of maturation [11]. Besides PSC, among TAG composition categories [7] there is also the positional-type composition (PTC). Because S were always virtually absent in the mid-position see above), the PTC was almost completely composed of only **SUS**, **SUU**, and **UUU** types, **SUU**, in most instances, greatly exceeding in its content (41–49 %) each of two other types.

Hierarchical Cluster Analysis

The dendrograms of an association of 13 *Euonymus* L. species based on the linkage distance and resulting from the

Table 3 Positional-species and positional-type (**SUS**, **SUU**, **UUU**) composition of TAG from mature fruit arils of the *Euonymus* section species, mol % of total TAG

TAG	*E. bunge-anus*[a]	*E. euro-paeus*[b]	*E. hamilto-nianus*[c]	*E. phello-manus*[d]	*E. semiex-sertus*[e]	*E. siebol-dianus*[f]
StLSt	0.1	0.1	–	0.7	0.1	0.1
StOP	0.2	0.2	0.1	0.9	0.1	0.3
StOO	0.1	0.2	0.5	0.4	0.1	0.3
POP	1.8	1.0	3.4	2.2	1.2	1.2
OOO	0.5	0.9	0.5	0.6	0.3	1.5
StLP	1.7	1.3	2.8	6.8	1.8	1.4
StLO	0.9	1.2	1.0	3.5	1.0	1.6
POO	1.9	1.9	2.5	2.2	1.3	2.8
StOL	0.1	0.2	0.3	0.4	0.1	0.2
PLP	13.8	6.6	17.8	17.3	14.6	6.0
POL	1.9	2.4	4.0	2.0	2.0	1.8
HOO	0.7	0.5	0.1	0.1	–	1.5
OOL	1.0	2.2	1.5	1.0	1.1	2.0
POH	1.3	0.5	0.2	0.2	0.1	1.4
StLL	0.9	1.5	1.7	3.1	1.5	1.0
PLO	14.5	12.4	13.5	17.5	15.8	13.4
OLO	3.8	5.8	2.6	4.4	4.3	7.5
PHP	–	0.2	0.5	–	–	0.6
OHO	–	0.2	0.1	–	–	0.7
PHO	–	0.4	0.4	–	–	1.2
StLH	0.6	0.3	0.1	0.3	–	0.8
OOLn	–	0.1	0.1	0.1	–	0.1
PLL	14.9	15.2	21.5	15.9	24.6	8.8
POLn	0.1	0.2	0.1	0.1	–	0.1
OLL	7.8	14.3	8.5	8.0	13.3	9.9
PLH	9.7	3.2	1.3	1.4	0.6	6.6
HLO	5.1	3.0	0.5	0.7	0.3	7.4
HOL	0.7	0.6	0.1	0.1	–	1.0
LOL	0.5	1.4	1.2	0.5	0.8	0.7
OHL	–	0.5	0.2	–	–	0.9
OLnO	0.1	0.3	0.1	0.1	0.1	0.2
PLnP	0.3	0.3	0.6	0.4	0.3	0.2
PLnO	0.4	0.6	0.5	0.4	0.3	0.3
PLnL	0.4	0.7	0.7	0.4	0.5	0.2
PHL	0.1	0.5	0.6	–	–	0.8
OLLn	0.4	0.9	0.3	0.6	0.3	0.3
LLL	4.0	8.7	6.5	3.6	10.4	3.2
HLL	5.2	3.7	0.8	0.7	0.5	4.9
PLLn	0.7	1.0	0.7	1.2	0.6	0.3
OLnL	0.2	0.6	0.3	0.2	0.3	0.3
HLH	1.7	0.4	–	–	–	1.8
LLnL	0.1	0.4	0.2	0.1	0.2	0.1
Sum	98.2	96.6	98.4	98.1	98.5	95.4
SUS	17.9	9.7	25.2	28.3	18.1	9.8

cluster analysis of these species, as regards (1) the PSC of their aril TAG (Tables 3, 4) and (2) the FA composition of *sn*-2 position of these TAG (Table 1), are shown in Fig. 1a, b. It is seen that all species belonging to the *Euonymus* subgenus formed a separate cluster (cluster 1) and, inside this cluster, the species belonging to the *Melanocarya* section

Table 3 Continued

TAG	*E. bunge-anus*[a]	*E. euro-paeus*[b]	*E. hamilto-nianus*[c]	*E. phello-manus*[d]	*E. semiex-sertus*[e]	*E. siebol-dianus*[f]
SUU	48.5	42.4	49.6	49.0	48.5	41.6
UUU	31.8	44.5	23.6	20.8	31.9	44.0

TAG containing minor FA other than hexadecenoic acid positional isomers were not taken into account

"–" implies not found

[a] Also present were StOH, HOH, PLnH, OLnH, LLLn, HLLn, and LLnH (0.1–0.2 % of total TAG each)

[b] Also present were StHO, StLnP, StLnO, StLnL, StLLn, HHO, PHH, HOH, OLnH, LHL, LnOL, PLnLn, and LLnLn (0.1–0.4 % of total TAG each)

[c] Also present were StLnP, StHP, StLnL, StLLn, LnOL, and LHL (0.1–0.2 % of total TAG each)

[d] Also present were StOSt, StLnP, StLnO, StLnL, StLLn, and LnOL (0.1–0.2 % of total TAG each)

[e] Also present were PHH, PLnH, LHL, HLLn, and LLnH (≤0.1 % of total TAG each)

[f] Also present were StOH, StHO, StHL, StHP, StHH, PHH, HOH, HHO, PLnH, LHL, OLnH, HHL, HHH, LLLn, HLLn, and LLnH (0.1–0.7 % of total TAG each)

(*E. alatus* and *E. sacrosanctus*) formed, in their turn, a separate sub cluster (marked with dotted line in Fig. 1a).

All species in the clusters 2 and 3 except *E. pauciflorus* belonged to the subgenus *Kalonymus* (see table 1 in [8]). Up to now, *E. pauciflorus*, according to current classification [3], has been assigned to the *Pseudovyenomus* section (*Euonymus* subgenus), but, in compliance with modern evidence, it is more similar to representatives of the subgenus *Kalonymus* not only in its aril TAG composition but also in the anatomical structure of its mature arils [14]. Therefore, in the present study and in the previous one [6], *E. pauciflorus* was included in cluster 2, enclosing, along with cluster 3, the species from the *Kalonymus* subgenus.

Based on the results of clustering which operated with 42 (Table 3) and 40 (Table 4) positional species of TAG, we analyzed the variance of average concentration of each positional species of TAG between clusters and selected a population of 12 *i*-th species. Their calculated concentrations were characterized by maximal variability. Using this species population, the analysis of *k*-means was performed. It is seen (Table 5) that, at hierarchical cluster analysis, the association of separate euonymus species into independent clusters was affected by only 12 positional species comprising ≤64.5 % of the total TAG. Intercluster differences of *k*-means of concentrations of these species were significant at various levels of statistical confidence. So, the species of the *Euonymus* subgenus (cluster 1), as compared to the representatives of the *Kalonymus* subgenus (clusters 2 and 3), were characterized by significantly lesser levels of TAG positional species having one, two, and three O residues (POP, OOO, POL, StOO, StOP, and OOL) and by significantly higher concentrations of species with two and three L residues (PLL and LLL).

As for the *Melanocarya* section, it differed from the *Euonymus* section in a 2.3-fold higher OOO concentration and twice higher OOL content, an increased level of POL, POO, and LLL, and a lower PLP concentration. These differences failed to be confirmed by *k*-means analysis, but were sufficient for isolating *E. alatus* and *E. sacrosanctus* species as a separate subcluster by hierarchical analysis (see above).

Similar results were obtained when using this analysis not only for PSC but also for the values of FA content in *sn*-2 position of TAG from Table 1. However, in the latter case (Fig. 1b), as distinct from the dendrogram based on PSC, the *Euonymus* species were associated into clusters at significantly lesser linkage distances. Thus, in Fig. 1b, these distances were 21–82 (cluster 1) and 37.5–106 (clusters 2 + 3), while in Fig. 1b they were 2–50 (cluster 1) and 7.9–120 (cluster 2). Moreover, in Fig. 1a, b, the intercluster differences became nonsignificant at the linkage distances of ~245 and 330, respectively, pointing to a greater precision of clustering in the latter case. Finally, clustering based on the FA composition of *sn*-1,3 positions of TAG from Table 1 failed to yield statistically significant results.

It is concluded that, as compared to the FA composition of total TAG [6], the PSC of TAG, as well as FA composition of their *sn*-2 position, much more closely corresponded to the taxonomy of euonymus species, and what is more, not only on the level of a subgenus but even on the level of a section.

Principal Component Analysis

At the same time, it was of interest to reduce, if possible, the number of variables (concentrations of TAG positional species) to only two factors grouped together according to the presence of O and L in *sn*-1, -2, or -3 positions of TAG and to obtain, in such common factor space, the distribution of *Euonymus* L. species compatible with their taxonomic position in this genus and similar to that shown in Fig. 1. In order to test this suggestion, we applied principal component analysis (PCA). Starting with the data of Tables 3 and

Table 4 Positional-species and positional-type (**SUS**, **SUU**, **UUU**) composition of TAG from mature fruit arils of several sections and species of the genus *Euonymus* L. (Table 1), mol % of total TAG

TAG	*E. alatus*[a]	*E. sacro-sanctus*[b]	*E. pauci-florus*[c]	*E. lati-folius*[d]	*E. macro-pterus*[e]	*E. maximo-wiczianus*	*E. sachali-nensis*[f]
StOSt	–	–	0.3	0.1	–	0.1	0.6
StLSt	0.1	0.1	0.4	–	0.1	0.1	0.3
StOP	0.4	0.3	2.8	1.1	1.6	2.0	6.2
StOO	0.3	0.2	2.9	2.1	0.4	1.0	5.1
POP	2.1	1.6	5.9	4.6	12.7	9.9	15.1
OOO	1.3	2.0	6.5	18.6	0.7	2.6	10.5
StLP	1.7	1.3	3.4	0.5	2.8	2.8	2.8
StLO	1.4	1.5	3.6	1.0	0.7	1.5	2.3
POO	3.4	3.5	12.4	18.5	6.0	10.2	25.1
StOL	0.4	0.3	1.0	0.5	0.4	0.7	0.1
PLP	8.9	7.2	7.3	2.2	23.0	14.2	6.8
POL	4.1	3.0	4.4	4.3	7.2	7.3	1.9
OOH	–	0.2	0.4	0.9	–	0.2	0.3
OOL	3.3	3.4	4.7	8.7	1.7	3.8	1.6
POH	0.1	0.1	0.4	0.5	0.2	0.4	0.4
StLL	1.7	1.3	1.3	0.2	0.8	1.0	0.2
PLO	14.1	16.2	15.4	9.0	11.0	14.7	11.4
StLnP	0.1	0.1	0.1	0.1	0.3	0.1	–
StLnO	0.1	0.1	0.1	0.2	0.1	–	–
OLO	5.6	9.1	8.1	9.0	1.3	3.8	4.7
StLH	0.6	0.1	0.1	–	–	0.1	–
OOLn	–	0.2	0.2	1.2	0.1	0.1	0.3
PLL	17.2	13.7	5.5	2.1	13.1	10.4	0.9
POLn	0.3	0.2	0.2	0.6	0.6	0.3	0.4
OLL	13.6	15.5	5.8	4.2	3.1	5.4	0.7
PLH	0.2	0.6	0.5	0.2	0.4	0.6	0.2
HLO	0.2	0.7	0.6	0.5	0.1	0.3	0.1
HOL	0.1	0.1	0.2	0.1	0.1	0.1	–
LOL	2.0	1.4	0.8	1.0	1.0	1.3	0.1
OLnO	0.3	0.6	0.3	1.3	0.1	0.1	0.1
PLnP	0.5	0.5	0.3	0.3	2.1	0.4	0.1
PLnO	0.7	1.0	0.5	1.3	1.0	0.4	0.2
PLnL	0.9	0.9	0.2	0.3	1.2	0.3	–
OLLn	0.9	1.0	0.3	0.6	0.2	0.2	0.2
LLL	8.3	6.6	1.0	0.5	1.9	1.9	–
HLL	0.2	0.6	0.2	0.1	0.1	0.2	–
PLLn	1.2	0.9	0.3	0.3	1.0	0.4	0.2
OLLn	0.7	1.0	0.2	0.6	0.3	0.3	–
LOLn	0.3	0.2	0.1	0.3	0.2	0.1	–
LLnL	0.4	0.4	–	0.1	0.2	0.1	–
Sum	97.7	97.7	98.7	97.7	97.8	99.4	98.9
SUS	13.8	11.1	20.5	8.9	42.6	29.6	31.9
SUU	46.7	43.6	48.8	41.1	44.1	49.3	48.4
UUU	37.2	43.0	29.4	47.7	11.1	20.5	18.6

TAG containing minor FA other than hexadecenoic acid positional isomers were not taken into account

[a] Also present were PHO, OHL, StLnL, StLLn, PHL, PLnLn, HLLn, LLnLn, and LLnH (0.1–0.2 % of total TAG each)

[b] Also present were StLnL, StLLn, PLnLn, OLnLn, and LLnLn (0,1 % of total TAG each)

[c] Also present were StOH, and StLLn, (0.1 % of total TAG each)

[d] Also present were PHP, PHO, OHO, StOLn, StOH, OHL, PHL, OLnH, and OLnLn (0.1–0.4 % of total TAG each)

[e] Also present were PHP, PHO, StLnL, StLLn, PHL, and PLnLn (0.1–0.3 % of total TAG each)

[f] Also present were StOH, and StOLn (0,1 % of total TAG each)

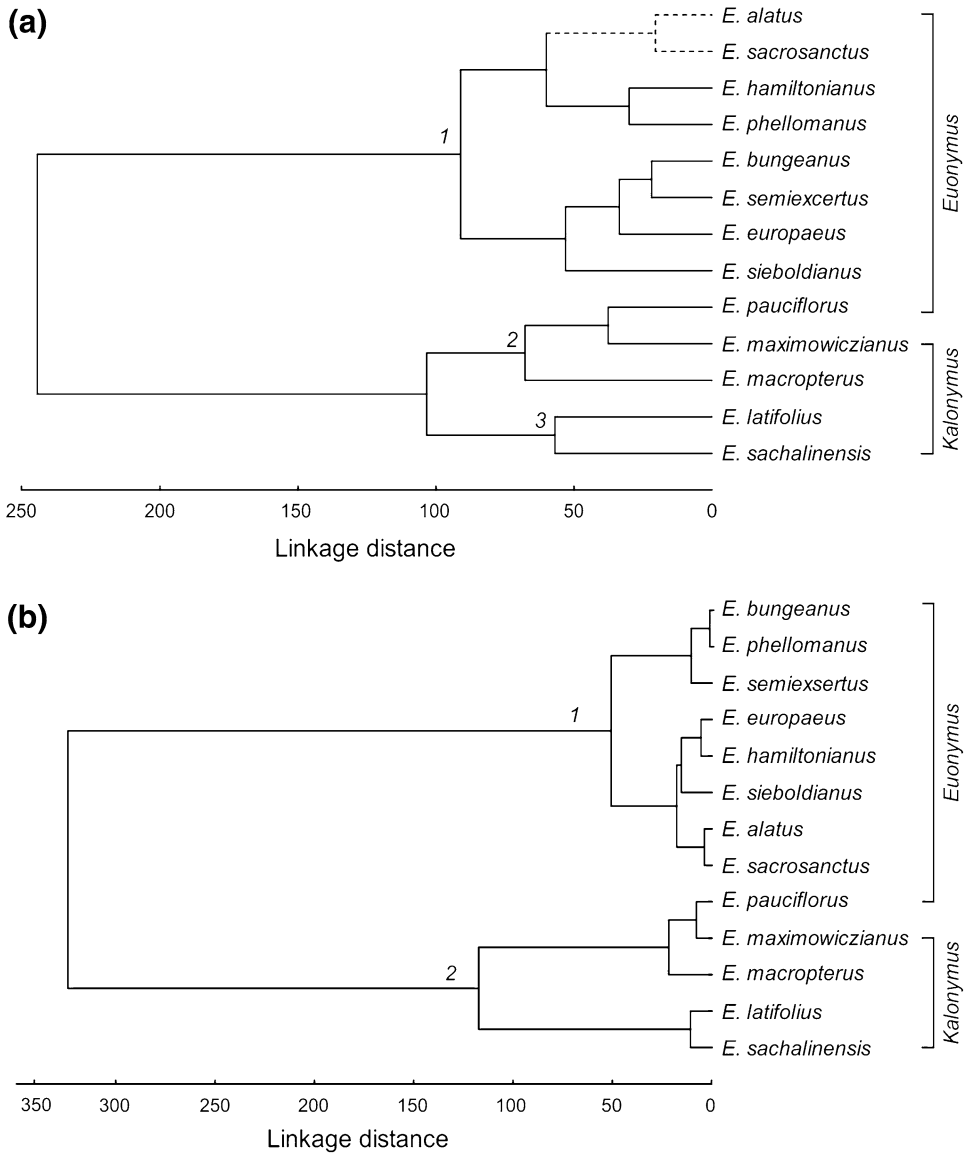

Fig. 1 Dendrograms of the association of 13 euonymus species into clusters based on the linkage distance and resulting from the cluster analysis of these species as regards **a** the PSC of their aril TAG (Tables 3, 4); and **b** the FA composition of *sn*-2 position of these TAG (Table 1); associated by Ward's method and city-block (Manhattan) metric

4, we formed the factor 1 grouping together the POP, OOO, StOP, StOO, POO, and OOLn species devoid of L, as well as the factor 2, which included the PLP, POL, StOL, OOL, LLP, and LLL positional species containing L.

An analysis by application of the scree test showed that the two factors formed here were sufficient for characterizing interspecies variation in the PSC of TAG inside the *Euonymus* L. genus; the eigenvalues plot (scree plot) was omitted. Factors 1 and 2 explained 98 % of the variance, 86.13 % accounting for by factor 1, and 11.87 % by factor 2.

Figure 2 demonstrates the PCA plot of component weights of separate *Euonymus* L. species in the common factor space according to their factor loadings brought about by the PSC of TAG. One can see that, in this plot, various species of the *Euonymus* subgenus formed well-separated groups near to main axes. One of these groups comprising *Euonymus* section species (except *E. europaeus*) was displaced toward positive values along both factors, while another (*Melanokarya* section species) was shifted into the region of their negative values. The projections of factor loadings brought about by the PSC of TAG from two *Kalonymus* subgenus species were situated at considerable distances from each other and from the principal axes.

These results suggested that factors 1 and 2 can serve as categorical variables for constructing classification trees and so to be used an auxiliary device for botanical and taxonomical investigations of the *Euonymus* L. genus. At the same time, to further justify this suggestion based as yet on a sample of only 13 euonymus species, more experimental evidence will be necessary.

Table 5 *k*-Means values of concentrations of *i*-th TAG positional species inside the clusters 1, 2, and 3, mol %, and statistical confidence of difference (α) between the concentrations of a given positional species in various clusters at the degree of freedom $df_1 = 2$ and $df_2 = 10$

TAG species	Clusters (Fig. 1a)			Significance
	1	2	3	
POP	1.6	10.8	7.6	***
OOO	0.6	1.5	10.8	
POL	2.3	7.1	4.0	
StOL	0.2	0.6	0.7	
StOO	0.2	0.7	3.1	
POO	1.7	7.3	17.0	
PLL	15.9	11.8	3.0	
LLL	6.2	2.0	0.5	
StOP	0.3	1.7	3.0	**
OOLn	0.1	0.1	0.6	
PLP	11.0	18.1	4.9	*
OOL	1.4	2.8	5.2	
Sum	41.5	64.5	60.4	

*** $\alpha < 0.005$, $F > 9.427$

** $\alpha < 0.01$, $7.559 < F < 9.426$

* $\alpha < 0.05$, $4.103 < F < 7.558$

TAG Positional Species Characteristics of Definite *Euonymus* L. Genus Taxonomic Units

Application of both clustering analysis and PCA demonstrated that there were statistically significant differences between various taxonomic units of *Euonymus* L. genus as regards the PSC of their aril TAG. Now, from the data of Table 1, it was calculated that, in total TAG of *Euonymus* subgenus and of *Kalonymus* subgenus (including *E. pauciflorus*), the median quantitative L/O ratio was 2.12 and 0.61, respectively. Accordingly, the TAG of *Euonymus* (Table 3) and *Melanocarya* (Table 4) sections were characterized by a predominance of only *sn*-2-L positional isomers: PLP, PLO, OLO, PLL, OLL, PLH, and LLL (up to 22 mol % each); in its turn, the *Melanocarya* section differed from the *Euonymus* one in a somewhat higher concentration of OOO, OOL, POL, POO, and LLL, as well as in a lower PLP content (see above).

At the same time, the TAG of the *Kalonymus* subgenus were marked by a prevalence of *sn*-2-O isomers, namely POP, OOO, POO, POL, and OOL (up to 25 mol % each; Table 4). Correspondingly, these subgenera were significantly (at $\alpha = 0.05$ %) distinct in the rate of incorporation of O and L residues in the *sn*-2 position. From Table 2, it was calculated that, in *Euonymus* and *Kalonymus*, the values of enrichment factor (EF_O) were, respectively, 0.70 ± 0.07 and 1.39 ± 0.10; selectivity factor (SF_O) $= 0.49 \pm 0.04$ and 0.88 ± 0.06; and the ratio $EF_L/EF_O = 2.88 \pm 0.20$ and 1.48 ± 0.32. Moreover, the *Kalonymus* subgenus significantly (at $\alpha = 0.1$) exceeded the *Euonymus* one in the content of **SUS**, while in the levels of **SUU** and **UUU** no statistically significant differences between these subgenera were found.

Previously, we have found that the *Euonymus* section species markedly distinguished from those of the remainder sections of *Euonymus* L. genus by an enhanced content of hexadecenoic acids-containing TAG (H-TAG) [6]. The present study demonstrated that the *Euonymus* section species, in their turn, also differed from each other in the amount of H-TAG (Tables 1, 3). Thus, the TAG of *E. bungeanus,*

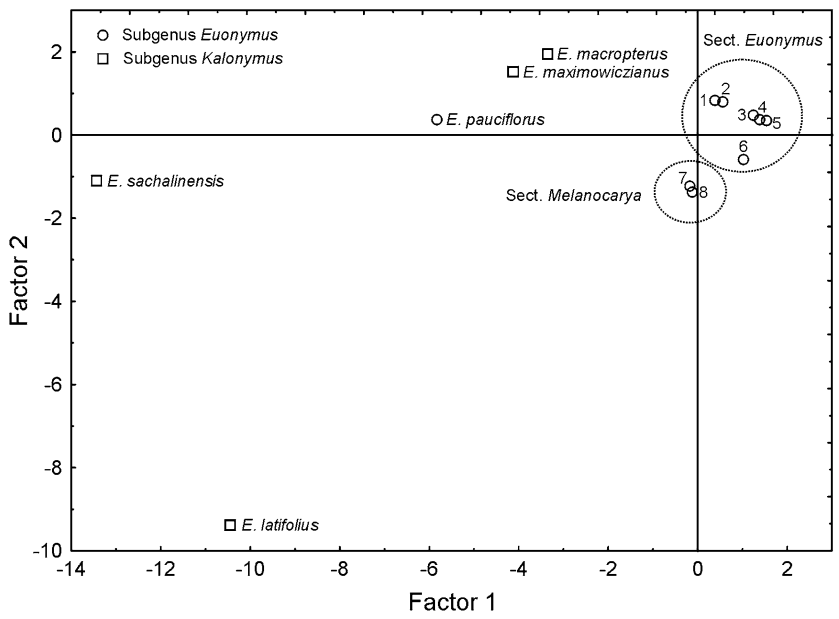

Fig. 2 PCA plot of component weights for *Euonymus* L. species (*1–8*) in a common factor space. *1*, *E. phellomanus*; *2*, *E. hamiltonianus*; *3*, *E. bungeanus*; *4*, *E. semiexsertus*; *5*, *E. sieboldianus*; *6*, *E. europaeus*; *7*, *E. alatus*; *8*, *E. sacrosanctus*

E. europaeus, and *E. sieboldianus* were characterized by a particularly enhanced content of H-TAG. In these species, the concentration of H-TAG in total TAG was no less than 29.1, 14.2, and 29 mol %, respectively. In **SUS**, H-TAG were virtually absent, and their content in the **SUU** and **UUU** of the above three species was 11.7, 17.4; 5.3, 8.9; and 10.9, 18.2 mol %, respectively; thus, the incorporation of H acyl groups in **UUU** TAG was much more active. As regards the stereospecificity of such incorporation, H was present mostly in extreme TAG positions, the level of *sn*-2-H TAG being only 0.1, 1.8, and 4.2 mol %, respectively. In *Melanocarya* (*E.alatus* and *E. sacrosanctus*), the content of H-TAG in total TAG did not exceed 1.4 and 2.3 mol %, respectively.

Similar distribution between mid- and extreme positions of H acyl groups in H-TAG was found in virgin olive oil [15], *Acer saccharum* and *A. saccharinum* seed oils [16], avocado mesocarp oil [17] and chufa (*Cyperus esculentus* L.) tuber oil [18]. At the same time, there exist oils with another type of H acyl group distribution. Particularly, in oils rich in H-TAG, close to uniform H acyl distribution with some prevalence of H acyl groups in the *sn*-2 position of TAG was established [12, 13, 19, 20]. In addition, these oils sharply differed from the *Euonymus* aril TAG in their affiliation with the positional types of H-TAG. For example, in sea buckthorn, hypanthium oil more than half of H-TAG belong to **SUU** type, 16.7 % to **SUS** type, 1.6 % to **SSU** type, and 0.9 % to **USU** type [19]; *Euonymus* aril oil is almost devoid of the last three types of H-TAG which are mostly belong to **SUU** and **UUU** types (Tables 3, 4).

In an earlier study, the TAG composition was investigated in the fruit oil of another representative of the Celastraceae family, *Celastrus paniculatus* [21]; however, in this instance, the seed oil TAG, rather than the aril oil used in our case, were employed. These TAG turned out to be characterized by a much higher concentration of S in their *sn*-2 position than the *Euonymus* aril ones, and their PTC was constituted of **SSS**, 4.1; **SSU**, 9.7; **USU**, 5.4; **SUU**, 40.1; **SUS**, 17.9; and **UUU**, 22.8 %. Therefore, further efforts are necessary for solving the problem of TAG composition of oils of Celastraceae plants.

Conclusion

The taxonomic classification of *Euonymus* L. genus was elaborated repeatedly, but up to now the infrageneric division and delimitation of many *Euonymus* species are still debatable [2]. The former classifications were based merely on the fruit and flower traits and other morphological characters [3, 4, 22]; however, recently, Li et al. [2] suggested some modifications to the existing phylogeny of *Euonymus* L. genus using DNA sequences of multiple nuclear and plastid markers. According to their study, *E. alatus* from the *Melanocarya* section of former taxonomic classifications belongs to *Kalonymus* section, *E. verrucosus* (formerly *Pseudovyenomus* section) to the *Euonymus* one, and *E. macropterus* (formerly *Kalonymus* section) should be attributed to the *Melanocarya* section [2]. The molecular sequence data allowed Li et al. [2] to make some other changes to *Euonymus* L. taxonomy system created earlier by Ma [3]. However, whereas our chemotaxonomy data are in good accordance with Ma's and other former taxonomy systems of *Euonymus* L. genus [3, 4, 22], they are contrary to the Li et al. suggestions. Therefore, the phylogeny of this genus needs further elaboration taking into account not only morphological characters but also DNA markers and, probably, characteristic properties of their lipid composition.

The representatives of *Euonymus* L. genus have a complex PSC of their aril TAG. Since *Euonymus* species belonging to different sections have PSC features distinguishing them from one another, these indices can be used for chemotaxonomic purposes. Another trait of the *Euonymus* species was the presence of up to 15 unusual minor FA including *cis*-vaccenic acid, Δ7-, Δ8-, and Δ11-hexadecenoic acids, Δ10-octadecenoic acid, etc., in their TAG and 3-acetyl-1,2-diacyl-*sn*-glycerols [6]. The structural analyses of molecular species of these neutral acylglycerols will be the subject of our future work.

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