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Comparison of the Triterpenoid Content of Berries and Leaves of Lingonberry *Vaccinium vitis-idaea* from Finland and Poland

Anna Szakiel,*[,†] Cezary Pączkowski,[†] Heini Koivuniemi,[‡] and Satu Huttunen[‡]

[†]Department of Plant Biochemistry, Faculty of Biology, University of Warsaw, ul. Miecznikowa 1, 02-096 Warszawa, Poland

[‡]Botany Division, Department of Biology, University of Oulu, P.O. Box 3000, 90014 Oulu, Finland

Supporting Information

ABSTRACT: Triterpenoid compounds extracted from fruits and leaves of lingonberry (*Vaccinium vitis-idaea* L.) collected in Finland and Poland were identified and quantitated by GC−MS/FID. The main lingonberry triterpenoid profile consisted of α-amyrin, β-amyrin, betulin, campesterol, cycloartanol, erythrodiol, fern-7-en-3β-ol, friedelin, lupeol, sitosterol, stigmasterol, stigmasta-3,5-dien-7-one, swert-9(11)-en-3β-ol, taraxasterol, urs-12-en-29-al, uvaol, oleanolic acid, and ursolic acid. To our knowledge, this is the first thorough description of triterpenoid compounds in this species. Ursolic acid was identified as a principal triterpene in lingonberry fruit. The influence of geographical origin on the level of individual triterpenoid compounds was examined, and considerable variations in triterpenoid profile between berries and leaves obtained from the two locations were observed. The most striking difference concerned the occurrence of fernenol and taraxasterol, which were found to be the major triterpenol in lingonberry leaves of Finnish and Polish origin, respectively.

KEYWORDS: *triterpenoids, lingonberry (Vaccinium vitis-idaea L.) fruit and leaf, GS−MS, oleanolic acid, ursolic acid, fernenol, taraxasterol*

INTRODUCTION

Triterpenoids, polycyclic compounds derived from the linear hydrocarbon squalene, are secondary plant metabolites ubiquitously distributed throughout the plant kingdom and distinguished by their remarkable structural diversity and numerous biological activities. In pharmacological tests, this important group of phytochemicals has been shown to display antiinflammatory, antiulcer, antimicrobial, hepatoprotective, immunomodulatory, hypolipidemic and cholesterol-lowering, antiatherosclerotic, wound-healing, anticoagulant, and anticarcinogenic properties.[1,2] These compounds are very promising as multitargeting agents in the treatment of certain cancers and inflammatory diseases,[3] as well as microbial infections, the latter being of special importance due to the fact that conventional drugs, which usually target only a single process of the infective cycle of parasites, favor the appearance of resistant mutants.[4] An exponential increase in the number of reports regarding bioactive triterpenoids over the past decade reflects the growing interest in their potential pharmaceutical, nutraceutical, and cosmeceutical applications. A number of these compounds is currently under development for use in new functional foods, drugs, cosmetics, and healthcare products. Therefore, natural triterpenoid-rich sources, particularly those derived from edible and medicinal plants, are the subject of much study.[5]

Triterpenoids are found in plants in a free form, but also as esters and glycosidic conjugates called saponins. These forms differ in their polarity and water-solubility, and, consequently, they are accumulated in different plant organs and cellular compartments. Compounds of low polarity in free and esterified forms are found in abundance in stem bark or surface cuticles, including those of edible fruits and leaves. Therefore, triterpenoids occurring in fruits are frequently lost as a result of fruit peeling or processing into products like juice. Consequently, small berries with edible peel, consumed fresh or after processing of the entire fruits into jam or sauce, are of special interest because they may be regarded as a rich natural dietary source of bioactive triterpenoids.

Recent research has firmly established that the dietary intake of berry fruits has a positive and profound impact on human health.[6] Much progress has been made in identifying phytochemicals present in these fruits, although bioactive triterpenoids remain among the less well-characterized compounds as compared to other groups of secondary metabolites, such as berry phenolics. The health benefits of plant-based foods are now thought to be largely dependent on phytochemicals acting in an additive and synergistic way,[7] and from this point of view, berries can be regarded as a functional mixture of water-soluble compounds including many antioxidants occurring in the fruit flesh, and lipophilic constituents present in the cuticle.

There have been numerous reports on phenolic compounds occurring in berries of *Vaccinium vitis-idaea* L. (lingonberry, cowberry);[8−13] however, the triterpenoid profile of this plant has not been fully investigated.[14] Lingonberry, a small evergreen shrub found in the herbaceous layer of circumboreal forests and Arctic tundra, is one of the most significant wild berry producers in countries of Northern and Central Europe, as well as Russia and Canada, and its berries are classed as a "superfruit", being particularly rich in antioxidants.[12] Lingonberry fruits and also the leaves of this plant play a well-

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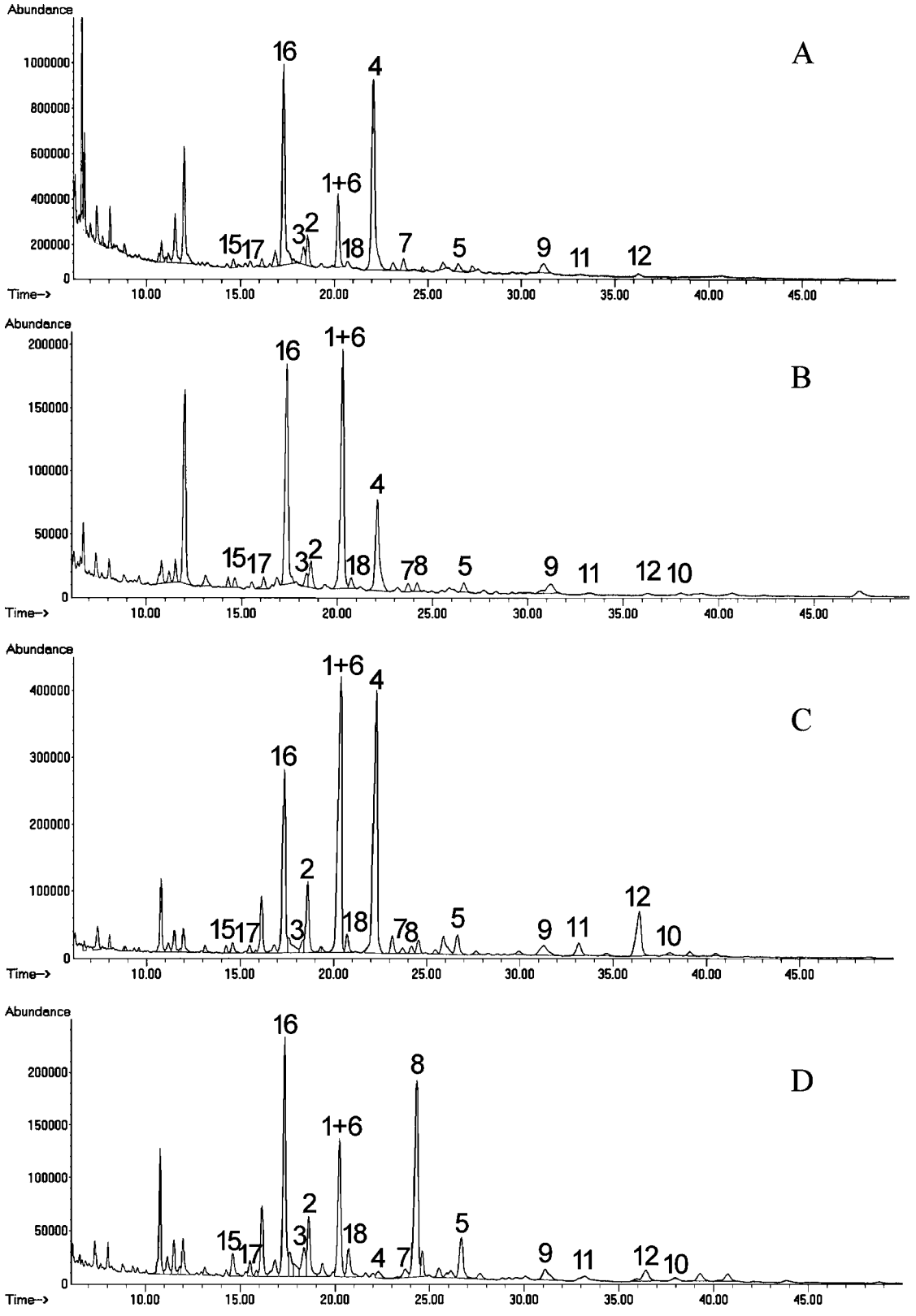

Figure 1. GC-FID chromatograms of the fractions containing sterols and neutral triterpenes (alcohols, aldehydes, and ketones) obtained from diethyl ether extracts of lingonberry fruits (A, Finnish; B, Polish) and leaves (C, Finnish; D, Polish). Peaks are numbered according to Figures 3 and 4.

established role in pharmacognosy, being widely used in herbal medicine, mainly to counteract urinary- and digestive-tract infections. The aim of the present study was to identify the main triterpenoid compounds occurring in lingonberry fruits and leaves. Moreover, because qualitative and quantitative differences in the contents of plant secondary metabolites have been related to the place of plant origin (i.e., local geoclimatic conditions), a comparison of triterpenoid profiles of

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lingonberry plants harvested in Finland and Poland was conducted.

MATERIALS AND METHODS

Plant Material. Lingonberry fruits and two types of foliage, young immature, current-year leaves and old, previous-year leaves, were collected from typical natural forest habitats in Finland (65°066 N; 25°458 E) and Poland (52°455 N, 21°332 E) in late August (berries and leaves) and December 2010 (leaves only). Late August is the starting time of the lingonberry fruit harvest in Finland, and the middle of the harvest season in Poland. The Finnish habitat, a typical mid/north-boreal forest in northern Ostrobothnia, is a mixed forest dominated by Scots pine *Pinus sylvestris* and some Norway spruce *Picea abies*, with *Vaccinium myrtillus*, *V. vitis-idaea*, and *Rhododendron tomentosum* as the main plants of the understorey. The Polish habitat, typical for the central Mazovia region, is a dry forest dominated with Scots pine, with *V. myrtillus*, *V. vitis-idaea*, and mosses in the undergrowth. Because of the difference in latitude, the two habitats differ in the length of the vegetative season, the photoperiod, and the thickness of snow cover in winter. In both forests, three collection sites were chosen, placed 250 m apart in an area of about 1 ha. At every site, 10 ramets were selected within a few meters of each other and transported to the laboratory, where fresh, healthy leaves were detached from the shoots, mixed, and weighed. Berries were collected directly from the plants in the forest. Young, current-year leaves were recognized by their position at the top of the stem and their bright green color, while the previous-year leaves were those growing just below the young leaves. The replicate 2 g samples of both berries and leaves were prepared from three different aggregate sets of 10−15 g. The Finnish samples were allowed to gently dry at room temperature in paper bags and were then sent by courier to the Laboratory of Plant Biochemistry in Warsaw. Finnish and Polish voucher specimens were deposited, respectively, in the herbaria of the University of Oulu (accession nos. OULU 10004161−10004166) and the University of Warsaw (accession no. WA 0000017596).

Chemicals and Standards. All solvents used for extraction and analysis were of analytical grade. Authentic standards of α-amyrin and ursolic acid methyl ester were purchased from Roth (Karlsruhe, Germany); β-amyrin, lupeol, uvaol, oleanolic acid, campesterol, sitosterol, and stigmasterol were purchased from Sigma-Aldrich (Steinheim, Germany). Faradiol (used to total recovery evaluation) was obtained in the Laboratory of Plant Biochemistry, University of Warsaw.

Extraction and Fractionation. Fruit and leaf samples (2 g, three replicates each) were dried at 60 °C, powdered, and extracted with diethyl ether (100 mL) in a small Soxhlet apparatus for 8 h. Extracts were evaporated to dryness at 40 °C under reduced pressure (extract masses were 14−16, 23−25, and 39−44 mg for fruits, young, and old leaves, respectively), and separated by preparative TLC (thin layer chromatography) on 20 cm × 20 cm glass plates coated with a 0.25 mm layer of silica gel 60H (Merck) in the solvent system $CHCl_3$/MeOH (97:3, v/v) into fractions of triterpene acids, free (nonesterified) neutral triterpenes (alcohols, aldehydes, and ketones) and sterols, and esters. The individual fractions were localized on plates by comparison with standards of oleanolic acid, sitosterol, and α-amyrin, visualized by spraying the relevant part of the plate with 50% H_2SO_4, followed by heating with a hot-air stream. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols were directly analyzed by GC−MS, fractions containing triterpene acids were methylated with diazomethane, and fractions containing triterpenoid (triterpene and sterol) esters were subjected to alkaline hydrolysis. The average recovery of α-amyrin, uvaol, stigmasterol, and ursolic acid methyl ester from praparative TLC plates was 98.6%, 97.2%, 98.9%, and 96.1%, respectively.

Derivatization of Triterpene Acids. Nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpene acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether, and held at 2 °C for 24 h.[14,15]

Alkaline Hydrolysis. The ester fraction was subjected to alkaline hydrolysis with 10% NaOH in 80% MeOH at 80 °C for 3 h. Subsequently, 5 volumes of water was added to each hydrolysate, the pH was neutralized with 5% CH_3COOH, and the obtained mixtures were extracted with diethyl ether (3 × 10 mL). These extracts were fractionated by preparative TLC as described above, and then fractions containing free triterpene alcohols and sterols were directly analyzed by GC−MS, while triterpene acid fractions were methylated prior to this analysis.

Identification and Quantification of Triterpenoids by GC−MS. An Agilent Technologies 7890A gas chromatograph coupled with a 5975C mass spectrometric detector was used for qualitative and quantitative analyses. Samples (dissolved in 1−4 μL of a 5:1 diethyl ether: methanol mixture) were applied by split injection 1:10. The column used was a 30 m × 0.25 mm i.d., 0.25 μm, HP-5MS (Agilent Technologies). Helium was used as a carrier gas at a flow rate of 1 mL/min. The following parameters were employed: column temp 280 °C, inlet and a flame ionization detector (FID) temp 290 °C, MS transfer line temp 275 °C, quadrupole temp 150 °C, ion source temp 230 °C, EI 70 eV, m/z range 33−500, FID gas (H_2) flow 30 mL/min (hydrogen generator), air flow 400 mL/min. Individual compounds (presented in Figures 3 and 4) were identified by comparing their mass spectra with library data from Wiley, 9th ed. and NIST 2008 Lib. SW (Version 2010), and, where available, by comparison of their retention times and corresponding mass spectra with those of authentic standards. Quantification was based on calibration curves prepared for the most typical representatives of each triterpenoid group (α-amyrin, uvaol, oleanolic acid methyl ester, sitosterol). The total recovery of known quantities of the triterpene dihydroxyalcohol faradiol (not present in lingonberry; retention time 40.0 min), added to the dried samples of plant material prior to their extraction, was 96%.

Separation of α-Amyrin and Lupeol by HPLC. Chromatographic analyses of samples containing neutral triterpenes including the mixture of α-amyrin and lupeol were performed in a Shimadzu (Japan) chromatographic system equipped with two LC-10AT pumps, CTO-10AS oven, and SPD-10A spectrophotometer set at 200 and 254 nm. The column used was a 250 mm × 4.6 mm i.d., 4 μm, Synergi MAX-RP 80A (Phenomenex, U.S.). All data were acquired and processed with Shimadzu CLASS-VP (V 5.032) chromatography data system software. The mobile phase (flow-rate 0.6 mL/min) was 100% acetonitrile (isocratic system), and separation was performed at 30 °C.

RESULTS AND DISCUSSION

Triterpenoid Profile. Representative GC-FID chromatograms of the fractions containing sterols and neutral triterpenes (alcohols, aldehydes, and ketones) obtained from diethyl ether extracts of lingonberry fruits and leaves are shown in Figure 1. In the fractions obtained from extracts of Finnish and Polish fruits (chromatograms A and B, respectively), the principal peaks of retention time (t_R) 17.3 and 22.2 min (numbered as 16 and 4) had mass spectra of sitosterol and fern-7-en-3β-ol, respectively, whereas the peak of t_R 20.2 min (numbered as 1 + 6) was associated with the mixture of two triterpene alcohols, α-amyrin and lupeol. The column employed for GC did not permit the separation of these two compounds. The identification of α-amyrin and lupeol as a mixture was confirmed by GC−MS analysis of their authentic standards, examined separately or combined together. The presence of both compounds was also revealed by HPLC analysis. The inability to separate two compounds by GC and/or HPLC is a recognized problem in triterpenoid identification.[16] Another significant peak in fruit extract chromatograms, of t_R 12.0 min, was identified as α-tocopherol. Smaller peaks were identified as campesterol (15), stigmasterol (17), cycloartanol (3), β-amyrin

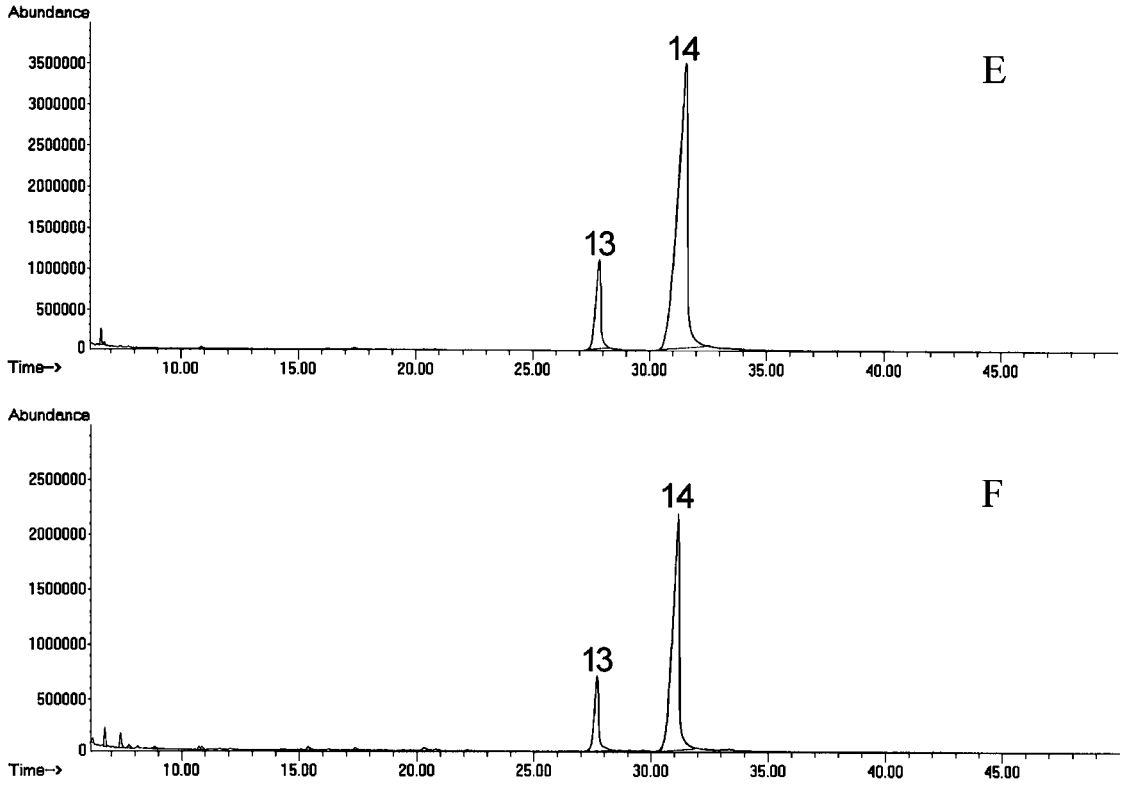

Figure 2. GC-FID chromatograms of the fractions containing methyl esters of triterpene acids from fruits of Finnish (E) and Polish (F) lingonberry: oleanolic acid methyl ester (13), ursolic acid methyl ester (14).

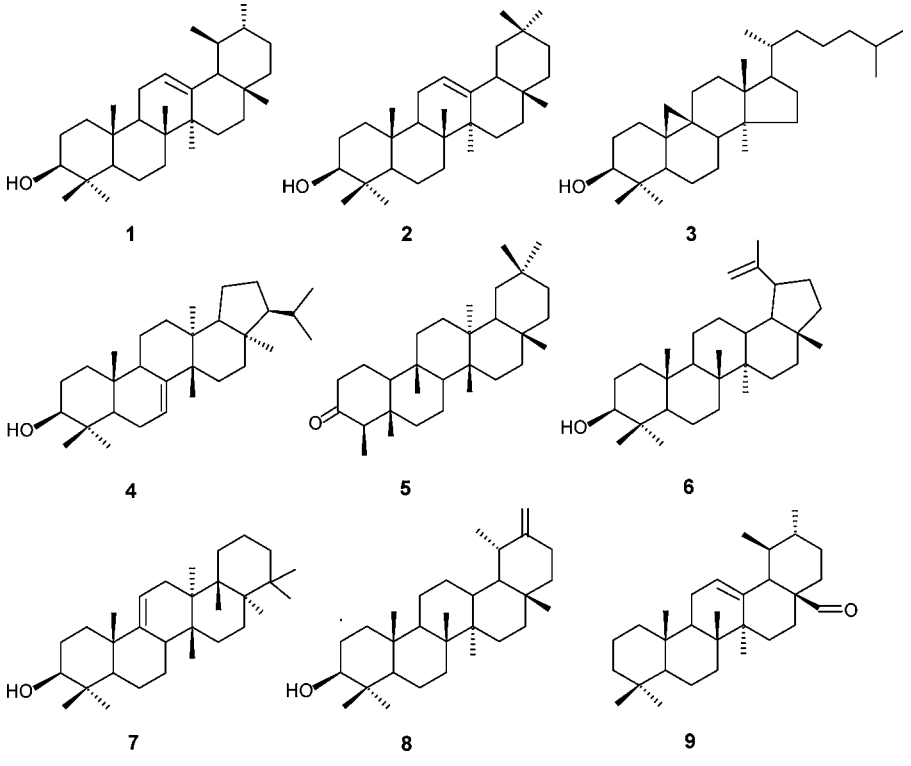

Figure 3. Chemical structures of triterpene monohydroxyalcohols, aldehydes, and ketones found in lingonberry fruits and leaves: (**1**) α-amyrin, (**2**) β-amyrin, (**3**) cycloartanol, (**4**) fern-7-en-3β-ol, (**5**) friedelin, (**6**) lupeol, (**7**) swert-9(11)-en-3β-ol, (**8**) taraxasterol, and (**9**) urs-12-en-28-al.

(2), stigmasta-3,5-dien-7-one (18), swert-9(11)-en-3β-ol (7), taraxasterol (8, only in chromatogram B), friedelin (5), urs-12-en-28-al (9), erythrodiol (11), uvaol (12), and betulin (10, only in chromatogram B).

In the leaf extract fractions containing sterols and neutral triterpenes (alcohols, aldehydes, and ketones), the main peaks detected in Finnish samples (Figure 1, chromatogram C) were those of sitosterol (16, t_R 17.3 min), α-amyrin/lupeol (1 + 6, t_R

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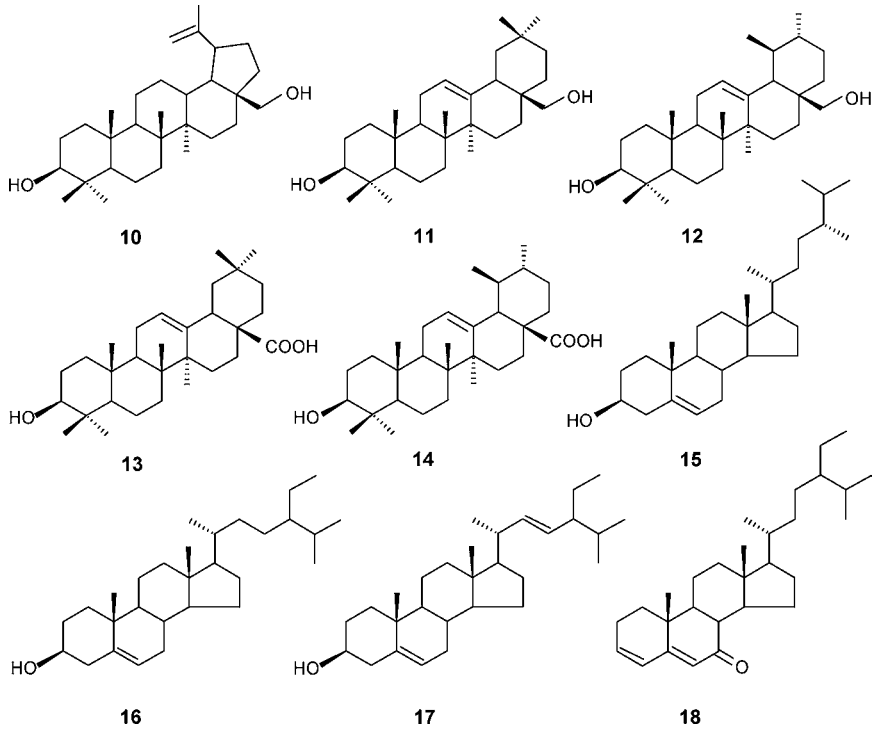

Figure 4. Chemical structures of triterpene dihydroxyalcohols, acids, and steroids found in lingonberry fruits and leaves: (**10**) betulin, (**11**) erythrodiol, (**12**) uvaol, (**13**) oleanolic acid, (**14**) ursolic acid, (**15**) campesterol, (**16**) sitosterol, (**17**) stigmasterol, and (**18**) stigmasta-3,5-dien-7-one.

20.3 min), and fern-7-en-3β-ol (4, t_R 22.2), whereas in Polish samples (Figure 1, chromatogram D) the main peaks (16, 1 + 6, and 8; t_R 17.3, 20.3, and 24.2 min, respectively) were associated with sitosterol, α-amyrin/lupeol, and taraxasterol. Another significant peak (t_R 10.8 min) occurring in both chromatograms was identified as the aliphatic long-chain alcohol, 1-octacosanol. Smaller peaks were associated with the same triterpenoids identified in fruit extracts.

Representative GC-FID chromatograms of the fractions containing methyl esters of triterpene acids are shown in Figure 2. Oleanolic and ursolic acid methyl esters (13 and 14), with respective t_R of 27.8 and 31.4 min, were identified in all extracts. The major peaks obtained for hydrolyzed triterpenoid esters (chromatograms not shown) were associated with sterols, while the minor peaks were those of triterpene acids and several triterpene alcohols (α-amyrin/lupeol, β-amyrin, cycloartanol, fernenol, taraxasterol, uvaol).

Thus, according to GC−MS analysis, the main triterpenoid profile of lingonberry is composed of two triterpene acids (ursolic and oleanolic); triterpene monohydroxyalcohols (α-amyrin, β-amyrin, cycloartanol, fernenol, lupeol, swertenol); triterpene dihydroxyalcohols (betulin, erythrodiol, and uvaol); one triterpene aldehyde (ursenal); one triterpene ketone (friedelin); plus steroids (campesterol, sitosterol, stigmasterol, and stigmastadienone). The chemical structures of these compounds are given in Figures 3 and 4. To our knowledge, this is the first thorough description of the triterpenoid profile of lingonberry.

Triterpenoid Content of Lingonberry Fruits. The results of quantitative determination of individual triterpenoids identified in Finnish and Polish lingonberry fruits are presented in Table 1. The total content was similar in fruits originating from both countries (differing only by 5%), and the two isomeric acids, oleanolic and ursolic, were the most abundant compounds, comprising 73% of all triterpenoids in Finnish, and 70% in Polish fruits. The level of triterpene acids was slightly higher (by 10%) in Finnish than in Polish berries, with respective oleanolic:ursolic acid ratios of 1:3.1 and 1:2.9.

More substantial differences were found in the amounts of other classes of triterpenoids in the two fruit samples. The fraction of the neutral triterpenes was more abundant (by 60%) in Polish than in Finnish berries. Moreover, the profiles of these compounds differed not only quantitatively but also qualitatively, because the triterpenols betulin and taraxasterol were found only in Polish fruits. The principal triterpenol in Finnish berries was fernenol (37% of the neutral triterpene fraction), followed by lupeol, α-amyrin, friedelin, and ursenal, which were present in similar quantities (approximately 10%). The quantification of α-amyrin and lupeol was based on the relative ratio of peak areas of these compounds separated by HPLC. In Polish berries, α-amyrin was predominant within this class of compounds (30% of the fraction), followed by lupeol (20%) and fernenol (19%). The triterpene ketone, friedelin, and the triterpene aldehyde, ursenal, were more abundant (by 30%) in Finnish berries, while triterpene dihydroxyalcohols, erythrodiol, and uvaol were found in relatively small amounts in both berry samples.

Finnish and Polish berries contained comparable amounts of free sitosterol, the dominant compound among phytosterols (83% and 75% of the steroid fraction in Finnish and Polish lingonberries, respectively). The amounts of campesterol and stigmastadienone were markedly higher in Polish fruits (1.5-fold and 2.5-fold, respectively), whereas the amount of stigmasterol was slightly lower (by 10%) in Polish than in Finnish berries. In turn, Finnish berries contained much higher levels of sitosterol and stigmasterol esters (2-fold and 10-fold, respectively), whereas the content of esters of triterpene compounds was similar in both fruit samples. Other steroids,

Table 1. Contents of Triterpenoids in Fruits of Lingonberry Collected in Finland and Poland in August 2010

compound (no.)	μg/g dry weight ± SD	
	Finnish berries	Polish berries
free:		
α-amyrin (**1**)	86.0 ± 4.0	392.3 ± 12.0
β-amyrin (**2**)	69.2 ± 1.9	95.6 ± 3.9
betulin (**10**)	n.d.	12.9 ± 1.3
cycloartanol (**3**)	60.3 ± 4.9	103.1 ± 4.4
erythrodiol (**11**)	2.5 ± 0.3	1.1 ± 0.1
fernenol (**4**)	294.9 ± 6.3	240.1 ± 14.8
friedelin (**5**)	85.1 ± 4.8	73.5 ± 3.0
lupeol (**6**)	90.9 ± 2.3	259.6 ± 3.4
swertenol (**7**)	20.1 ± 1.1	19.5 ± 1.4
taraxasterol (**8**)	n.d.	19.1 ± 1.7
ursenal (**9**)	87.5 ± 2.9	55.6 ± 3.1
uvaol (**12**)	3.5 ± 0.4	10.1 ± 0.9
sum of neutral triterpenes	800.0	1282.5
oleanolic acid (**13**)	2147.9 ± 76.8	2092.5 ± 77.4
ursolic acid (**14**)	6763.7 ± 88.6	6040.3 ± 103.1
sum of triterpene acids	8911.6	8132.8
campesterol (**15**)	101.1 ± 4.2	152.4 ± 3.4
sitosterol (**16**)	1327.9 ± 66.2	1357.0 ± 63.2
stigmastadienone (**18**)	96.7 ± 3.6	245.3 ± 7.1
stigmasterol (**17**)	65.3 ± 2.2	58.9 ± 3.3
sum of steroids	1591.0	1813.6
esters:		
α-amyrin	9.1 ± 0.8	30.1 ± 1.8
β-amyrin	tr	6.3 ± 0.2
cycloartanol	78.1 ± 3.5	25.5 ± 2.3
fernenol	24.2 ± 1.2	37.6 ± 2.9
taraxasterol	n.d.	n.d.
uvaol	tr	tr
oleanolic acid	1.3 ± 0.1	4.5 ± 0.5
ursolic acid	48.1 ± 1.7	8.5 ± 0.5
campesterol	n.d.	16.9 ± 1.5
sitosterol	580.9 ± 21.1	281.8 ± 20.0
stigmasterol	207.1 ± 13.6	20.7 ± 2.0
sum of esters	948.8	431.9
total	12 251.4	11 660.8

previously identified in lingonberry seeds,[17] including, for example, isofucosterol, lanostadienol, and citrostadienol, were not found in measurable quantities in the whole berry extracts.

The presented results show that the triterpenoid content in lingonberry fruit is relatively high, mainly due to the level of triterpene acids, which accounted for 0.9% and 0.8% of the dry weight of Finnish and Polish berries, respectively. Ursolic acid was found to be a principal triterpene compound in lingonberry fruit, as it is for some other edible berries of the genus *Vaccinium*, that is, *V. macrocarpon*, *V. oxycoccus*, and *V. corymbosum*.[18−20] However, this is not the case in bilberry *V. myrtillus*, where oleanolic acid is the predominant isomer.[19] Both of these acids are known for their antiinflammatory, anticancer, and antimicrobial activities, as well as for numerous other pharmacological properties,[21,22] and so they are likely to contribute to health benefits that may result from berry consumption. In contrast to cranberry (*V. macrocarpon*), lingonberry does not contain significant amounts of ursolic acid esters, such as *cis*- and *trans*-3-*O*-*p*-hydroxycinnamoyl esters, which have been reported to inhibit growth of prostate and breast tumor cells in vitro.[20,23,24] Despite this lack, the long list of diverse phytochemicals described in lingonberry indicates that it represents a good dietary source of bioactive phenolics and triterpenoids.

Triterpenoid Content of Lingonberry Leaves. Lingonberry plants possess two types of foliage: young, immature leaves growing on the upper part of the stems, and old, fully developed leaves located lower on the stems. These two leaf types are formed independently in consecutive growth seasons. The levels of plant secondary metabolites, including triterpenoids, can be influenced by changeable abiotic conditions (light, temperature, humidity), and so differences between leaves developed in separate years might be expected. Furthermore, Finland and Poland differ in the length of the vegetative season, and thus young, current-year leaves from these two locations should exhibit different rates of growth and biosynthesis of secondary metabolites.

The results of quantitative determination of individual triterpenoids identified in young and old lingonberry leaves collected in Finland and Poland are presented in Table 2 (leaves collected in August) and Table 3 (leaves collected in December). Generally, in August, young leaves from both countries contained less triterpenoids than old leaves (by 38% and 58% in Finnish and Polish plants, respectively), whereas the triterpenoid levels in old leaves collected from the two locations were very similar. The leaf content of the triterpene acids, oleanolic and ursolic, was significantly lower than that of berries (0.2% and 0.4% of dry leaf weight in young and old Finnish leaves, respectively; 0.3% and 0.5% in the corresponding Polish leaves). Ursolic acid was again more abundant, although it was less dominant than in berries, with respective oleanolic:ursolic acid ratios of approximately 1:2 and 1:2.2 in young and old Finnish leaves, and 1:2.1 and 1:2.2 in the corresponding Polish leaves.

In contrast to the case in berries, the neutral triterpenes were present in much higher amounts in leaves of Finnish than Polish plants, especially in young leaves collected in August (6-fold higher level). The most abundant compound of this class in Finnish leaves was fernenol (36% and 32% of this fraction in young and old leaves, respectively), which was found in only very small amounts in Polish leaves. The predominant triterpenol in Polish leaves was taraxasterol (32% and 43% of this fraction in young and old leaves, respectively), which in turn occurred in small amounts in Finnish leaves. In contrast to the triterpenoid profile of berries, detectable amounts of betulin were found not only in Polish, but also in Finnish leaves. The sterol fraction in leaves was much larger than in berries (almost 4-fold in leaves from both countries), again with sitosterol as the main compound.

The synthesis of triterpenoids appears to be particularly intensive in young leaves of Finnish plants during the summer. In young leaves sampled in Finland in late August, the level of triterpenoids reached 71% of that of equivalent leaves sampled in December, whereas in leaves of Polish plants the corresponding figure was only 54%, indicating sustained metabolic activity throughout the autumn, which accords with the longer growth season in Poland. This phenomenon is most notable in the level of triterpene alcohols and ketones, which increased 3.7-fold from August to December in young leaves of Polish plants.

In December, the triterpenoid content of young leaves accounted for 1.18% and 1.05% of dry leaf weight in samples from Finland and Poland, respectively, and the level of these compounds was slightly higher (by 13%) in the former. This

Table 2. Contents of Triterpenoids in Leaves of Lingonberry Collected in Finland and Poland in August 2010

	μg/g dry weight ± SD			
	Finnish plants		Polish plants	
compound	young leaves	old leaves	young leaves	old leaves
free:				
α-amyrin (**1**)	565.8 ± 7.4	539.8 ± 22.7	62.2 ± 2.2	286.6 ± 6.0
β-amyrin (**2**)	145.8 ± 6.2	115.0 ± 10.0	42.7 ± 2.1	171.9 ± 4.4
betulin (**10**)	13.1 ± 0.7	20.9 ± 2.8	30.9 ± 1.5	12.3 ± 0.6
cycloartanol (**3**)	82.3 ± 5.1	101.4 ± 8.3	37.3 ± 1.5	101.8 ± 3.1
erythrodiol (**11**)	45.3 ± 2.7	56.8 ± 3.6	2.4 ± 0.1	14.7 ± 0.4
fernenol (**4**)	839.2 ± 24.2	760.4 ± 13.8	13.3 ± 0.5	19.0 ± 0.9
friedelin (**5**)	77.9 ± 6.6	51.6 ± 3.2	4.1 ± 0.1	90.0 ± 2.1
lupeol (**6**)	299.6 ± 7.7	288.5 ± 8.9	43.8 ± 1.7	158.5 ± 3.8
swertenol (**7**)	35.4 ± 2.8	28.0 ± 0.9	17.8 ± 0.7	35.7 ± 0.8
taraxasterol (**8**)	28.3 ± 2.4	20.8 ± 0.7	130.3 ± 2.2	743.5 ± 5.9
ursenal (**9**)	57.4 ± 3.9	46.8 ± 1.7	12.9 ± 0.7	47.9 ± 0.7
uvaol (**12**)	159.4 ± 8.8	191.7 ± 6.7	4.3 ± 0.1	50.8 ± 1.3
sum of neutral triterpenes	2349.5	2221.7	402.0	1732.7
oleanolic acid (**13**)	808.4 ± 29.2	1386.4 ± 41.2	912.0 ± 10.8	1448.7 ± 27.3
ursolic acid (**14**)	1585.7 ± 52.7	3048.2 ± 73.3	1878.8 ± 40.3	3158.2 ± 91.5
sum of triterpene acids	2394.1	4434.6	2790.8	4606.9
campesterol (**15**)	100.7 ± 13.5	74.8 ± 4.3	48.6 ± 0.9	130.9 ± 1.2
sitosterol (**16**)	2368.0 ± 93.2	5427.2 ± 77.2	1992.3 ± 40.9	6194.4 ± 53.6
stigmastadienone (**18**)	552.2 ± 47.4	575.2 ± 16.2	139.9 ± 2.0	269.5 ± 16.6
stigmasterol (**17**)	62.4 ± 9.6	127.1 ± 11.5	30.7 ± 1.5	84.9 ± 2.8
sum of steroids	3083.3	6204.3	2211.5	6679.7
esters:				
α-amyrin	80.7 ± 7.4	88.7 ± 2.3	5.0 ± 0.1	9.8 ± 0.1
β-amyrin	27.6 ± 3.6	29.8 ± 1.1	1.6 ± 0.1	6.8 ± 0.2
fernenol	99.8 ± 9.2	73.3 ± 2.4	n.d.	n.d.
taraxasterol	n.d.	n.d.	9.0 ± 0.4	38.4 ± 0.7
uvaol	30.7 ± 2.7	n.d.	n.d.	n.d
oleanolic acid	8.9 ± 1.6	15.5 ± 0.6	10.7 ± 0.4	14.4 ± 0.3
ursolic acid	49.8 ± 4.6	56.0 ± 1.5	53.7 ± 1.3	56.4 ± 1.7
campesterol	56.1 ± 4.9	74.4 ± 2.2	7.6 ± 0.4	9.6 ± 0.3
sitosterol	235.2 ± 7.7	455.7 ± 12.2	229.9 ± 16.8	361.9 ± 22.3
stigmasterol	31.2 ± 3.1	39.9 ± 1.9	10.8 ± 1.1	20.4 ± 1.3
sum of esters	620.0	833.3	328.3	517.7
total	8446.9	13 693.9	5732.6	13 537.0

difference was even smaller in old leaves (6.5%), in which the respective amounts of triterpenoids reached 1.5% and 1.4% of dry leaf weight. Levels of triterpene acids, steroids, and triterpenoid esters were higher in old leaves collected from either country, suggesting that the synthesis of these classes of compounds is countinued in the following growth season. In contrast, the amount of neutral triterpenes was higher in young, current-year leaves than in old, previous-year leaves irrespective of their geographical origin, which may be explained by the influence of varying abiotic conditions in consecutive growth seasons, as discussed above, but could also be due to further decreases in the levels of these compounds as a result of their subsequent transformations (e.g., biosynthesis of acids from the respective amyrins), as well as possible esterification or glycosylation. Nevertheless, regardless of the leaf age, the most notable difference between the triterpenoid profiles was the identity of the major triterpene alcohol, fernenol in Finnish leaves and taraxasterol in Polish leaves.

The GC−MS/FID analysis of diethyl ether extracts obtained from dried fruits and leaves of lingonberry revealed a high content of triterpenoid compounds, the majority of which are present in a free, nonesterified, form. The mixture of two isomeric acids, ursolic and oleanolic, prevailed among the pentacyclic triterpenes occurring in the investigated lingonberry organs. However, the detailed lingonberry triterpenoid profile is highly complex, and, besides acids, it was found to comprise mono- and dihydroxyalcohols, one aldehyde and one ketone, with structures based on several types on skeletons. Of these, the lupane-, oleanane-, and ursane-type triterpenes are particularly highly valued for their pharmacological effects. As might be expected, several typical phytosterols and steroids were also detected in lingonberry fruits and leaves.

Various plant materials are currently being investigated as potential sources of materials for the nutraceutical, pharmaceutical, or cosmetic industries. Our results showed that both lingonberry fruit and leaves contain many potentially bioactive triterpenoid compounds and so might be considered as potential sources of triterpenoid-rich multipotent plant extracts, not only with regard to their most abundant constituents, that is, ursolic and oleanolic acids, which are rather ubiquitous in plants, but also for the other compounds they contain, such as amyrins, lupeol, erythrodiol, uvaol, taraxasterol, or fernenol. Fernenol, which has until now been obtained in substantial amounts mainly from ferns and several tropical plants, has been

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Table 3. Contents of Triterpenoids in Leaves of Lingonberry Collected in Finland and Poland in December 2010

	μg/g dry weight ± SD			
	Finnish plants		Polish plants	
compound	young leaves	old leaves	young leaves	old leaves
free:				
α-amyrin (**1**)	690.5 ± 12.2	390.8 ± 9.0	238.3 ± 8.6	197.7 ± 15.1
β-amyrin (**2**)	171.1 ± 6.8	145.8 ± 3.7	180.1 ± 5.3	128.1 ± 9.3
betulin (**10**)	14.1 ± 0.1	n.d.	tr	10.5 ± 1.0
cycloartanol (**3**)	130.9 ± 4.7	155.1 ± 5.8	22.0 ± 1.0	32.9 ± 1.7
erythrodiol (**11**)	66.5 ± 3.0	64.7 ± 2.2	20.4 ± 1.0	30.2 ± 1.8
fernenol (**4**)	1174.5 ± 41.6	793.3 ± 14.0	18.6 ± 0.2	13.8 ± 0.5
friedelin (**5**)	47.5 ± 1.3	40.6 ± 2.0	16.0 ± 0.9	85.3 ± 2.3
lupeol (**6**)	353.1 ± 9.8	196.0 ± 7.0	119.3 ± 2.6	79.4 ± 3.8
swertenol (**7**)	52.3 ± 1.9	63.90 ± 2.0	36.9 ± 1.5	20.0 ± 1.6
taraxasterol (**8**)	19.0 ± 0.5	32.0 ± 1.5	752.0 ± 5.5	557.4 ± 8.0
ursenal (**9**)	63.0 ± 0.9	44.2 ± 2.3	38.8 ± 1.6	64.6 ± 5.8
uvaol (**12**)	285.1 ± 6.2	259.3 ± 9.3	34.0 ± 0.9	109.2 ± 3.6
sum of neutral triterpenes	3067.6	2185.7	1476.4	1329.1
oleanolic acid (**13**)	1131.3 ± 57.0	1296.2 ± 37.7	1300.0 ± 73.5	1428.4 ± 63.0
ursolic acid (**14**)	2330.6 ± 59.8	2982.0 ± 93.3	2728.2 ± 85.2	2970.2 ± 83.6
sum of triterpene acids	3461.9	4278.2	4028.2	4398.6
campesterol (**15**)	207.4 ± 3.7	229.0 ± 7.5	142.8 ± 7.5	301.2 ± 6.1
sitosterol (**16**)	3645.1 ± 57,2	6283.7 ± 86.8	3866.2 ± 70.1	6530.2 ± 130.8
stigmastadienone (**18**)	623.1 ± 27.0	652.6 ± 14.2	224.3 ± 5.2	324.9 ± 16.4
stigmasterol (**17**)	71.4 ± 2.0	69.7 ± 0.9	108.9 ± 4.0	182.4 ± 11.3
sum of steroids	4547.0	7235.0	4342.2	7338.7
esters:				
α-amyrin	95.1 ± 2.4	104.0 ± 3.3	18.2 ± 0.6	24.0 ± 1.0
β-amyrin	24.3 ± 1.1	38.2 ± 0.7	8.9 ± 0.4	12.0 ± 1.7
fernenol	104.3 ± 3.4	99.9 ± 2.4	n.d.	n.d.
taraxasterol	n.d.	9.1 ± 0.4	62.5 ± 1.6	72.2 ± 2.1
uvaol	40.8 ± 1.9	6.5 ± 0.1	n.d.	10.2 ± 0.4
oleanolic acid	11.2 ± 0.8	16.4 ± 0.3	5.3 ± 0.2	10.6 ± 1.3
ursolic acid	75.6 ± 2.3	67.7 ± 1.5	35.7 ± 2.2	50.7 ± 3.2
campesterol	61.2 ± 1.5	81.5 ± 1.5	10.2 ± 0.7	13.2 ± 1.4
sitosterol	314.9 ± 12.8	830.6 ± 11.6	512.8 ± 9.0	791.5 ± 18.4
stigmasterol	36.5 ± 2.1	45.7 ± 0.7	14.2 ± 1.0	24.7 ± 2.0
sum of esters	763.9	1299.6	667.8	1009.1
total	11 840.4	14 998.5	10 514.6	14 075.5

reported to possess potent fungicidal activity against plant pathogens like *Collectotrichum gloeosporioides*, and thus may be considered for use as a natural fungicide for the control of anthracnose.[25] We found that significant amounts of this compound are present in the leaves of Finnish, but not Polish, lingonberry plants.

Indeed, the results of this study revealed significant differences in the quantitative profiles of triterpenoids occurring in fruits and leaves of lingonberry plants collected in Finland and Poland. Even though the total triterpenoid contents were not markedly different in the samples collected from the two sites, the amounts of some individual compounds varied considerably. Our recent studies have revealed that identified triterpenes are located mainly in fruit and leaf cuticular waxes, and, therefore, the observed differences in their content in Finnish and Polish plants, especially in leaves, are likely to be the consequence of adaptation to factors such as the growing season length or the thickness of snow cover in winter.[26] The current results confirm the strong dependence of the abundance of secondary metabolites on the geographical origin of the plant and highlight the relationship between the local geoclimatic conditions and the phytochemical content. Moreover, according to the observed compositional changes related to plant vegetative stage, the quality and quantity of plant natural products can be markedly different in the plants growing in the same location but harvested in succeeding years or in different seasons.

The reported fluctuations in the content of individual triterpenoids do not significantly impact on the nutritive value or the traditional usage of lingonberry in food products, and probably have a negligible effect on the health benefits resulting from lingonberry consumption. However, they should be carefully considered when the plant material is to be used for dietary supplements, functional foods, cosmetics, healthcare products, or any other purposes for which the properties of individual bioactive compounds are essential. The results presented here support the strong need for the evaluation and standardization of plant material to ensure its optimal quality and suitability for specific applications.

■ ASSOCIATED CONTENT

Supporting Information

Table showing retention times of identified triterpenes and steroids from lingonberry fruits and leaves. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +48225543316. Fax: +48225543221. E-mail: szakal@biol.uw.edu.pl.

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Notes

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