CHEMICAL CONSTITUENTS FROM WILD *Oxycoccus palustris* FRUIT FROM NORTH TYUMEN OBLAST

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The composition of cranberry extracts obtained from fresh fruit growing under natural conditions were studied using GC–MS. The principal constituents of wild cranberry collected from various sites of north Tyumen oblast were benzyl alcohol and α-terpineol and 2-methylbutyric, malic, citric, benzoic, and cinnamic acids in addition to fatty alcohols and acids.

Keywords: *Oxycoccus palustris*, organic acids, alcohols, aldehydes, esters, terpenes.

Cranberry continues to attract the attention of researchers because it exhibits a broad spectrum of therapeutic activity (capillary strengthening, anti-inflammatory, bactericidal, fungicidal, anticancer, wound-healing) that is due to the presence in its fruit of a complex mixture of biologically active compounds.

Many researchers have studied its acid and phenol composition [1–4].

The quantitative content of individual constituents changes as a function of habitat according to studies of the chemical composition [5, 6].

The chemical composition of cranberry fruit growing under natural conditions varies as a result of the combined natural and climate factors (heat and light, hydrothermal regime, atmospheric pressure, etc.) inherent to each region.

The question of qualitative changes of wild cranberry as a function of habitat within a single region is very interesting and little studied. Therefore, we analyzed cranberry extracts obtained from fresh fruit collected in different regions of north Tyumen Oblast by studying the natural compounds occurring in each sample.

We detected more than 50 components, many of which were easily identified during the study of the plant materials. Table 1 lists the relative concentrations of the compounds in percent of the total contained in the fruit.

The analysis of the extracts showed that the content of most compounds was less than 0.05% whereas several constituents were present in trace quantities. This may be due to the fact that many of the compounds are intermediate metabolites and are consumed during the synthesis of others. Thus, cinnamic aldehyde C_9H_8O and its alcohol $C_9H_{10}O$, which are present in the fruit, are intermediates in the biosynthesis of hydroxycinnamic and hydroxybenzoic acids, which in turn are involved in formation of phenols (flavonoids, anthocyanins, tannins, etc.). Malic $C_4H_6O_5$ and citric $C_6H_8O_7$ acids, which occur in cranberry, are formed from oxalic acid and its oxalates and are further utilized to form di- and tricarboxylic acids (Krebs cycle) [7]. 2-Methylbutyric acid $C_5H_{10}O_2$ with an isoprenoid skeleton was found among the volatile acids in cranberry fruit from the studied regions. Terpenes are known to be isoprene derivatives. Therefore, it is highly probable that 2-methylbutyric acid is one link in the synthesis of several terpenes.

Terpenes occur in essential oils and are responsible for the specific aroma of the plant. The synthesis of essential oil constituents begins in the very early development stages of the plant and reaches a maximum at the start of flowering. Most plant terpenes are secondary metabolites involved in plant protection. Thus, menthol and limonene act as repellents for insects. 1,8-Cineol has allelopathic action that has an inhibiting influence on surrounding plants [8, 9].

Ocimene $C_{10}H_{16}$ and limonene $C_{10}H_{16}$ were identified in cranberry growing in Tobol region. Ocimene is a precursor of many monoterpenes including limonene. α-Terpineol and eucalyptol (1,8-cineol) are formed from the latter.

The terpenoids α-terpineol ($C_{10}H_{18}O$) and camphor ($C_{10}H_{16}O$) were found in all studied plant samples. Eucalyptol ($C_{10}H_{18}O$) was identified in only the sample from Nizhnevartovsk region. Cranberry from this region contained also menthol ($C_{10}H_{20}O$) and farnesol ($C_{15}H_{26}O$), the latter being a sesquiterpene.

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TABLE 1. Chemical Composition of *Oxycoccus palustris* Fruit from Various Habitats

Compound	Retention time, min	Region		
		Nizhnevartovsk	Surgut	Tobol
		Aldehydes and ketones		
3-Hexen-2-one	2.99	0.2	1.0	0.7
Benzaldehyde	6.29	0.1	0.1	0.3
Acetophenone	9.11	1.1	1.3	0.5
Octanal	10.14	+	1.8	1.1
Decanal	13.02	+	0.2	0.7
Cinnamic aldehyde	15.01	+	–	+
Benzophenone	23.88	0.2	0.8	0.3
		Alcohols		
1-Hexanol-2-ethyl	7.92	0.5	1.0	2.5
Benzyl alcohol	8.17	0.5	0.1	0.5
Benzenemethanol, α,α-dimethyl	9.66	0.2	0.5	0.1
Ionol (4-methyl-2,6-di-*tert*-butylphenol)	20.84	1.2	1.0	+
Hydroxycinnamic alcohol	33.39	+	+	–
Oleyl alcohol	32.54	2.2	1.4	2.9
Cetyl alcohol	29.09	1.5	1.3	2.8
9,12-Octadecadien-1-ol	33.85	0.5	–	0.8
Eicosanol	32.99	1.5	1.6	2.2
		Terpenes		
Ocimene	5.47	–	–	+
Limonene	7.96	–	–	0.1
Eucalyptol	8.07	0.1	–	–
Camphor	11.41	1.7	10.0	2.3
Menthol	12.20	+	–	0.1
α-Terpineol	12.72	0.5	0.7	0.9
Edulan	32.89	0.4	0.4	+
Farnesol	44.62	0.5	0.2	0.3
Campesterol	53.51	3.1	1.6	0.7
β-Sitosterol	53.99	0.6	–	–
		Acids		
2-Methylbutyric acid	3.53	+	0.1	0.3
Benzoic acid	11.90	6.2	11.8	15.0
n-Decanoic acid	17.26	0.6	+	0.8
Cinnamic acid	18.86	0.2	0.2	+
Malic acid	14.95	2.1	3.9	0.2
Citric acid	19.45	–	0.8	0.3
Myristic acid	26.55	0.7	–	0.4
Palmitic acid	30.68	5.0	1.0	6.3
Oleic acid	33.95	0.5	–	2.5
Stearic acid	34.39	4.2	0.4	7.0
Linoleic acid	34.47	0.3	–	1.7
		Esters		
Diethyloxalate	2.80	–	0.3	–
Butylacetate	3.16	0.2	0.9	1.6
Ethyl-2-methylbutyrate	3.71	0.1	–	0.2
Ethylmethoxylevulinate	9.64	0.4	–	–
Ethylbenzoate	12.01	27.3	20.1	22.7
2-Ethylhexylbutanoate	16.14	0.3	0.5	1.0
Butylbenzoate	17.62	+	–	+
Ethylcinnamate	19.97	0.4	0.7	0.2
Diethylmalate	14.64	0.6	0.3	0.1
Anethole (isoestragol)	15.30	0.3	+	0.1
Ethylmyristate	27.29	–	–	0.5
Isopropylpalmitate	27.93	0.3	0.5	1.0
Ethylpalmitate	31.29	0.6	–	0.9
Ethylstearate	34.95	2.2	+	0.4

+, Component content <0.05%;–, component absent.

Mono-, di-, and sesquiterpenes are widely distributed in plants but, as a rule, in small concentrations. They are precursors in biosynthetic pathways to tri- and tetraterpenes.

Campesterol and β-sitosterol (only in the Nizhnevartovsk sample), which are unsaturated sterols, were found in the plant materials. Campesterol and β-sitosterol are the basic structural components of cell membranes. It was noted [10, 11] that these compounds are D provitamins.

Furthermore, edulan $C_{13}H_{20}O$ (CAS# 41678-29-9) was identified in cranberry. Compounds of this type can be formed during the biosynthesis of carotinoids and are formally oxidized fragments of these compounds [12].

Cranberry contained the fatty acids caproic, myristic, palmitic, oleic, stearic, linoleic, and arachic. Furthermore, ethyllinoleate $C_{22}H_{38}O_2$ was identified in the extracts (samples No. 1 and 2) in addition to esters of the aforementioned acids. These chemical compounds are unsaturated essential fatty acids and are considered vitamin-like biologically active compounds [13].

The fatty alcohols are metabolites of fatty acids and were also identified in wild cranberry. Oleyl, cetyl, and tetracosanyl alcohols were observed in fruit from all sites. Nizhnevartovsk cranberry contained 9,12-octadien-1-ol.

Aldehydes, ketones, and alcohols are involved in biochemical transformations; have unique aromas; and as a whole impart to cranberry its unique taste and aroma. Benzaldehyde was identified in the berries and has the pure aroma of bitter almond. Acetophenone and benzophenone were also present in the plant samples and have pleasant fruity aromas.

Many esters found in cranberry also contribute to the taste and aroma (Table 1). Furthermore, the cranberry extracts contained derivatives of phenol such as anethole (CAS# 104-46-1) and ionol (CAS# 128-37-0, BHT). Anethole, which is the ester of phenolpropene and methanol, is also called anise camphor because it smells like anise in its pure form. Anethole was identified in all cranberry samples. Ionol was present in berries collected near Nizhnevartovsk and Kogalym. These compounds are anti-oxidants. They most likely fulfill a protective role from unfavorable environmental factors in plants. Ionol is also a starting material in the biosynthesis of other biologically active compounds [14].

Thus, a comparison of the chemical composition of extracts obtained from wild plants collected from different habitats in western Siberia (Nizhnevartovsk, Surgut, Tobol) showed that the qualitative composition did not change substantially with the exception of individual constituents. This is consistent with a genetically constant property of wild cranberry to synthesize a certain set of organic compounds.

EXPERIMENTAL

Plant material was collected in natural habitats during full fruiting (end of September to the start of October 2008).

Ground cranberry fruit was extracted at room temperature by aqueous alcohol (40% mass fraction alcohol) and then by $CHCl_3$ (3×) at two different pH values (10 and 2). The combined $CHCl_3$ fractions were dried over anhydrous Na_2SO_4. The resulting samples were studied by GC–MS in a Perkin–Elmer Clarus 500 MS gas chromatograph with a quadrupole mass-selective detector. We used an SE-54 30-m glass column of internal diameter 0.25 mm and statioary phase thickness 0.25 mm and He carrier gas at constant flow rate 0.5 mL/min. The temperature was programmed from 80 to 280°C at heating rate 10°C/min. The vaporizer temperature was 270°C. Mass spectra were recorded at ionizing potential 70 eV. Qualitative analysis was performed by comparing full mass spectra of the constituents with the appropriate data of pure compounds and our own specialized library. The quantitative content of the berry constituents was calculated from the peak areas in the gas chromatograms without using correction coefficients.

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