CHEMICAL COMPOSITION OF Ledum palustre

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The leaves of Ledum palustre (crystal tea ledum) are used in medical practice as an expectorant. In order to study the active substances of this plant, we have extracted the total substances from the plant with liquefied carbon dioxide and Khladon-11 [~Freon 11], which have come into ever wider use recently for the extraction of plant substances.

On chromatographing a CO_2 extract of crystal tea ledum we isolated a hydrocarbon with the composition $C_{28}H_{50}$, a hydrocarbon with the composition $C_{10}H_{16}$, identified as myrcene [1, 2], an ester compound with the composition $C_{60}H_{116}O_2$; a sesquiterpene alcohol with the composition $C_{15}H_{26}O$, identified as palustrol [1-3]; a sesquiterpene alcohol with the composition $C_{15}H_{26}O$ identified as ledol [1, 2, 4]; a monohydric alcohol with the composition $C_{23}H_{44}O$; a flavonoid with the composition $C_{18}H_{16}O_5$, identified as 5-hydroxy-4',7-dimethoxy-6-methylflavone [5]; a sesquiterpene ketone with the composition $C_{15}H_{22}O$; a dihydric alcohol with the composition $C_{36}H_{60}O_2$; and an alcohol with the composition $C_{24}H_{40}O_2$.

We also isolated all these substances from the khladon extract. Of the substances listed, only myrcene, palustrol, and ledol have previously been described for crystal tea ledum [1-4]; this is the first time that the others have been isolated.

EXPERIMENTAL

For extraction we took pharmacopial raw material (mixture of leaves and small branches) of crystal tea ledum collected in August in the Kostroma oblast, containing 1% of essential oil. To find the optimum conditions we first performed experimental extractions in a laboratory apparatus and selected the conditions for performing the process: the time, the ratio of raw material and solvent, the degree of comminution of the raw material, and the temperature.

On extraction with liquefied carbon dioxide, the maximum yield of extract was 2.9% (from raw material comminuted in a crusher and on rolls to a particle size of 5-10 mm, the ratio of raw material and solvent 1:25; temperature 20°C; pressure $2.4 \cdot 10^4$ Pa; time of extraction 180 min). On extracting the raw material with Khladon-11, the maximum yield of extract was 9.24%. The extracts obtained consisted of greenish yellow oily masses. The amounts of essential oil in the extracts were 15 and 15.9%, and in the meal 0.4 and 0.44%, respectively.

Attempts to isolate individual substances from the extract by dissolution both in nonpolar and in polar solvents did not give the desired results, and after this the extracts were chromatographed on a column of KSK silica gel (1:30) and were eluted first with petroleum ether (bp 70-100°C) and then with mixtures of petroleum ether and diethyl ether in various ratios and with diethyl ether.

The substances obtained were dried over P_2O_5 in a vacuum pistol with heating by ethanol vapors and without heating (depending on the melting points of the substances): crystalline substances were dried for 2 h and noncrystalline substances for 6 h.

The IR spectra (mulls in paraffin oil) were taken on a UR-10 spectrophotometer, the UV spectra (solutions in 96% ethanol) on a Hitachi EPS-3T spectrophotometer, the NMR spectra on a Varian JNM-4H-100 MHz spectrophotometer in $CDCl_3$ solution, and the mass spectra on a Varian CH-8 spectrometer.

The results of the microanalysis of all the substances isolated corresponded to the calculated figures.

Isolation of a Hydrocarbon with the Composition $C_{28}H_{50}$. Fraction 1 of the petroleum-ether eluates of a CO_2 extract of crystal tea ledum yielded a hydrocarbon in the form of lustrous colorless scales with mp 62-64°C (from petroleum ether); composition $C_{28}H_{50}$; M^+ 386.

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IR spectrum: no absorption bands of C=O and OH groups.

PMR spectrum, δ, ppm: singlet at 1.2 ($-CH_2$ and $-C-CH_3$), triplet at 0.85 ($-CH_2-CH_3$).

Isolation of Myrcene. Fraction 11 of the petroleum-ether eluates from a CO_2 extract of crystal tea ledum yielded a hydrocarbon in the form of a colorless mobile liquid with bp 47°C (5 mm), n_D^{20} 1.4700; composition $C_{10}H_{16}$. The IR and NMR spectra of the hydrocarbon isolated and of myrcene were identical.

Isolation of an Ester Compound with the Composition $C_{60}H_{116}O_2$. Petroleum ether–diethyl ether (9:1) eluted an ester compound in the form of white granules, mp 65-67°C, composition $C_{60}H_{116}O_2$.

IR spectrum: ν_{max} 1740 cm^{-1} (OCO).

NMR spectrum, δ, ppm: two triplets of 2 H each at 4.1 and 2.2 ($-CH_2-C(=O)-OCH_2-$); triplet at 0.8 (CH_2-CH_3); singlet at 1.2 ($-CH_2$ and $C-CH_3$).

Isolation of Palustrol. Petroleum ether–diethyl ether (8:2) eluted a sesquiterpene alcohol in the form of a colorless viscous liquid with bp 115-117°C (1.5 mm), n_D^{20} 1.4890; composition $C_{15}H_{26}O$.

The IR and NMR spectra of the substance isolated and of palustrol were identical.

Isolation of Ledol. Petroleum ether–diethyl ether (7:3) eluted colorless crystals, mp 104-106°C (from ethanol); composition $C_{15}H_{26}O$.

The IR and NMR spectra of the sesquiterpene alcohol isolated and of ledol were identical.

Isolation of a Monohydric Alcohol with the Composition $C_{23}H_{44}O$. Petroleum ether–diethyl ether (7:3) eluted colorless whorled crystals, mp 74-76°C (from petroleum ether–diethyl ether); composition $C_{23}H_{44}O$; M^+ 336.

IR spectrum: ν_{max} 3280-3310 (OH).

NMR spectrum, δ, ppm: triplet at 3.6 (CH–OH), singlet at 1.2 (CH_2–and $C-CH_3$), triplet at 0.9 (CH^2-CH_3).

Isolation of 5-Hydroxy-4',7-dimethoxy-6-methylflavone. Petroleum ether–diethyl ether (1:1) eluted yellow needles with mp 184-186°C (from ethanol); composition $C_{18}H_{16}O_5$; M^+ 312.

IR spectrum, ν_{max}, cm^{-1}: w 3050 (OH with chelate bond), a 1670 (C=O), 1630, 1600, 1580 (C=C).

NMR spectrum, δ, ppm: two doublets at 7.80 (J=8 Hz) – $H_{2'}$ and $H_{6'}$ and at 6.95 (J=8 Hz) – $H_{3'}$ and $H_{5'}$; two singlets at 3.87 and 4.02 – two OCH_3; singlet at 2.10 – $CH_3-C=C$.

UV spectrum: λ_{max} in ethanol 277 and 330 nm; with sodium methanolate 287 and 322 nm; with sodium acetate 277 and 330 nm; with sodium acetate and boric acid 277 and 332; with aluminum chloride 279 and 337 nm; with aluminum chloride and hydrochloric acid 288 and 343 nm.

Isolation of a Sesquiterpene Ketone with the Composition $C_{15}H_{22}O$. Petroleum ether–diethyl ether in a ratio of 1:1 yielded a thick yellowish viscous liquid with the composition $C_{15}H_{22}O$. IR spectrum, ν_{max}, cm^{-1}: 1690 (CO–C=C) and 1620 (C=C).

The study of the structure of this compound will be the subject of a separate paper.

Isolation of a Dihydric Alcohol with the Composition $C_{36}H_{60}O_2$. Ethereal eluates yielded whorled crystals with mp 220-221°C (from ethanol); composition $C_{36}H_{60}O_2$.

IR spectrum, ν_{max}, cm^{-1}: 3340-3360 (OH).

NMR spectrum, δ, ppm: signal at 5.2 (CH=C–), two quartets at 3.8 and 3.4 (two CH_2OH), singlet and doublets in the 0.8-1.1 region ($CH_3-\underset{|}{C}-$; $CH_3-\underset{|}{CH}-$).

Isolation of an Alcohol with the Composition $C_{24}H_{40}O_2$. The ethereal eluates yielded white whorled crystals with mp 169-171°C (from ethanol), with the composition $C_{24}H_{40}O_2$.

IR spectrum, ν_{max}, cm^{-1}: 3350-3280 (OH).

NMR spectrum, δ, ppm: quartet at 3.75 (CH_2OH), singlets and doublets in the 0.6-1.7 region ($CH_3-\overset{|}{\underset{|}{C}}-$; $CH_3-\underset{|}{CH}-$).

SUMMARY

Extraction of the herb Ledum palustre with liquefied carbon dioxide and Khladon-11 has yielded 10 individual substances belonging to various classes and various groups of organic compounds: hydrocarbons, alcohols, ketones, monoterpenes, sesquiterpenes, and flavonoids.

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SOME BACTERICIDAL PROPERTIES OF OLIGOPEPTIDES AND REGULAR POLYPEPTIDES INCLUDING LYSINE AND ORNITHINE RESIDUES

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Histones take part as repressors in the regulation of gene activity, which is determined by their chemical nature: a high content of lysine and arginine. It is possible that the appreciable bactericidal activity of histones in relation to various microbes of the coli group and micrococci is also connected with this circumstance [1]. The antimicrobial mechanism of the action of histones has not yet been studied, but in view of their polycation-exchange properties it may be assumed that they are capable in some way of deforming membrane structures in microorganisms and of being nonspecific repressors.

Still more pronounced bactericidal properties in relation to certain bacteria are possessed by poly(α-amino acid)s containing lysine and arginine residues, and also their copolymers. Thus, copolymers containing ornithine, lysine, and arginine residues are active compounds not only for E. coli but also for Staphylococcus aureus [1]. The most active known antibacterial copolymer is a copolymer of leucine and ornithine in a ratio of 1:1. Its activity is 5 μg/ml [2]. The polypeptide H-(Leu-Orn-Leu)$_n$-OH possesses a similar activity [2].

The impression has been created that bactericidal properties depend on the primary structure of the polypeptide. To investigate this question we have obtained a series of oligopeptides containing lysine residues and have used regular polypeptides obtained previously, the synthesis of which has been described elsewhere [3-5]. The minimum concentrations of the oligo- and polypeptides possessing antibacterial properties are given below, all the other polypeptides possessing antibacterial activity at concentrations greater than 10 mg/ml:

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