CHEMICAL CONSTITUTION OF *Ledum palustre*

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UDC 543+547.9+633.88

From the herb *Ledum palustre* L., collected in July in Kostroma province we have isolated β-sitosterol, the triterpenoids taraxerol, uvaol, and ursolic acid, the coumarin fraxetin, the known flavonoids quercetin, hyperoside, and 3,3',7-tri-O-methylquercetin, and a new natural flavonoid. The latter was isolated in the form of light yellow crystals with mp 188-190°C (from ethanol), with the composition $C_{19}H_{18}O_8$. On the basis of IR, UV, NMR, and mass spectroscopy the structure of 4',5-dihydroxy-3,3',5',7-tetramethoxyflavone has been suggested for it.

We have previously [1] given information on the identification of substances obtained from *Ledum palustre* L. (crystal tea ledum) by liquefied gases [CO_2 and khladon-11 (Freon-11)]. In the present paper we give information on the identification of the substances obtained by organic solvents.

On extraction with organic solvents, in addition to the substances described previously [1] we isolated β-sitosterol, the triterpenoids taraxerol, uvaol, and ursolic acid, the coumarin fraxetin, and four flavonoids, two of which (quercetin and hyperoside) have been described previously for crystal tea ledum [2] and two have not (we have provisionally called them F-I and F-II). We shall dwell in more detail on the identification of these substances.

Substance F-I was obtained in the form of light yellow crystals with mp 147-149°C (from ethanol) having the composition $C_{18}H_{16}O_7$. Its IR spectrum had absorption bands at 3270 cm^{-1} (OH), 1665 cm^{-1} (CO—C=C), and 1600, 1500, and 1470 cm^{-1} (C=C—C C). The UV spectrum had two absorption maxima of equal intensity at 258 and 365 nm showing that these substances belonged to the flavonol group.

When the hydroxy groups were ionized with sodium methanolate, a bathochromic shift of the long-wave maximum by 43 nm with no reduction in intensity was observed, which indicates the presence of an OH group at C_4' together with a substituted OH group at C_3. Ionization by sodium acetate caused no shift in the maximum of the bands and, therefore, there is no free OH group at C_7. Bathochromic shifts of the long-wave and shortwave maxima by 45 and 15 nm on the addition of aluminum chloride showed the presence of a free OH group at C_5.

The presence of two OH groups in the molecule of F-I was confirmed by the preparation of a diacetate $C_{22}H_{20}O_9$, mp 175-177°C (from ethanol).

The NMR spectrum of the TMS ether of F-I contained the signals of three methoxy groups (signal at 3.8 ppm), and the signals of the protons of methoxy groups in the NMR spectrum of F-I in pyridine-d_5 were observed in the form of three singlets in the same region. Thus, in the F-I molecule there are two hydroxyls at C_5 and C_4' and three methoxy groups which are situated at C_3, C_7, and C_3', as follows from the NMR spectrum of the initial substance and of its acetate: two doublets in the 6.25 and 6.5 ppm regions, which are the signals of the H_6 and H_8 protons; a split 1 H signal at 6.9 ppm, H_5'; and a 2 H signal at 7.45-7.75 ppm, H_2' and H_6' [3].

The results obtained permit us to suggest the structure of 4',5-dihydroxy-3,3'-7-trimethoxyflavone for the substance isolated. On comparing the structure of the substance with those described in the literature, we observed its identity with the structure of a flavonoid isolated previously which was called 3,3',7-tri-O-methylquercetin [4]. Later, a substance of the same structure was described by other workers; however, neither the first nor the second authors gave the physical constants of the substance but only its spectral characteristics. Thus, the flavonoid that we have isolated is 3,3',7-tri-O-methylquercetin.

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 175-180, March-April, 1980. Original article submitted December 3, 1979.

0009-3130/80/1602-0131$07.50

TABLE 1

Absorption bands	λ_{max}, nm										
	C_2H_5OH	$+CH_3ONa$		$+AlCl_3$		$+AlCl_3$ $+HCl$		$+CH_3COONa$		$+CH_3COONa$ $+H_3BO_3$	
	λ	λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ
I	365	415	+50	415	+50	415	+50	365	0	365	0
II	255	267	+12	280	+25	280	+25	255	0	255	0

Flavonoid F-II was isolated in the form of light yellow crystals with mp 188-190°C (from ethanol) having the composition $C_{19}H_{18}O_8$. Its IR spectrum contained absorption bands at 3510 cm^{-1} (OH), 1660 cm^{-1} (CO—C=C), 1610, 1590, and 1570 cm^{-1} (C=C—C=C). Characteristics of its UV spectrum permit the substance isolated to be allocated to the flavonol group, since it has two maxima of equal intensity at 255 and 365 nm. Analysis of the UV spectra with ionizing additives (Table 1) showed the presence of two hydroxy groups in positions C_4 and C_5 (bathochromic displacement of the maxima on the addition of sodium methanolate and of aluminum chloride, respectively).

The presence of two hydroxy groups in the F-II molecule was shown by the preparation of a diacetate having the composition $C_{23}H_{22}O_{10}$, mp 221-222°C (from ethanol). The NMR spectrum of the TMS ether of F-II had the signals of four methoxy groups in the 3.8 ppm region in the form of several overlapping singlets, and in the NMR spectrum of F-II in pyridine and the NMR spectrum of its acetate the signals of the protons of the methoxy groups were partially resolved and appeared in the same region in the form of three singlets with intensities of 3 H, 6 H, and 3 H.

Thus, the F-II molecule has been found to contain two hydroxyls — one at C_5 and the other at C_4' — and four methoxyls the positions of which were determined on the basis of the NMR spectra of the TMS ether of F-II and of its acetate (Fig. 1). In the 6.12-6.35 ppm region there are two doublets of 1 H each, H_6 and H_8, and there is a 2 H singlet at 7.30 ppm, H_2' and H_6'. The results obtained permit the assumption for the substance isolated of the structure of 4',5-dihydroxy-3,3',5',7-tetramethoxyflavone. This substance has not been described in the literature.

The mass spectrum of F-II has the peak of the molecular ion M^+ with m/e 374 (100%) and the other main ions are $(M - H)^+$, $(M - CH_3)^+$, $(M - H_2O)^+$, $(M - HCO)^+$, $(M - H_2CO)^+$, $(M - OCH_3)^+$, and $(M - CH_3 - CO)^+$, the formation of the $(M - CH_3 - CH_3OH)^+$ fragment with m/e 327 (25%) can be explained by the ortho effect of methoxy and hydroxy groups in the $(M - CH_3)^+$ ion with m/e 359 (81%). The mass spectrum of the diacetate of F-II shows the peak of the molecular ion with m/e 458 (20%), indicating the presence of two acetyl groupings in the molecule and confirming the presence of two hydroxy groups in compound F-II. The main ions are: $(M - 2CH_2CO)^+$, $(M - CH_2CO - CH_3CO)^+$, and $(M - 2CH_2CO - CH_3)^+$. The formation of strong peaks in the mass spectrum of the acetyl derivative with m/e 416 (43%) and 374 (86%), corresponding to the loss of one or two molecules of ketene, confirms the presence of two hydroxy groups of the phenolic type in the F-II molecule. The appearance of strong peaks of the fragments $(M - CH_2CO - CH_3CO)^+$ with m/e 373 (35%) and $(M - 2CH_2CO - CH_3)^+$ with m/e 359 (100%) shows the ortho arrangement of the methoxy and acetoxy groups. Thus, the dissociative ionization of flavonoid F-II is in harmony with the suggested structure.

EXPERIMENTAL

The compositions of the substances obtained were determined from their percentage C and H contents and M^+ in the mass spectrum.

TLC was carried out on Silufol plates, the spots being revealed with a 0.5% solution of $KMnO_4$ in 0.5% H_2SO_4. NMR spectra were recorded on a JNM-4H-100 MHz spectrometer and mass spectra on a CH-8 instrument with an ionizing voltage of 75 V.

Extraction with Petroleum Ether. The comminuted pharmaceutical raw material (mixture of leaves and small twigs) of crystal tea ledum collected in July in the Kostroma oblast (1 kg) was steeped in petroleum ether at room temperature for one day. Extraction was repeated three times, and the extract was evaporated and the residue was chromatographed on silica gel L 100/250 μ. Petroleum ether and mixtures of petroleum ether and diethyl ether (9:1, 8:2,7:3) eluted a hydrocarbon, a wax, palustrol, ledol and 5-hydroxy-4'7-dimethoxy-6-methyl-

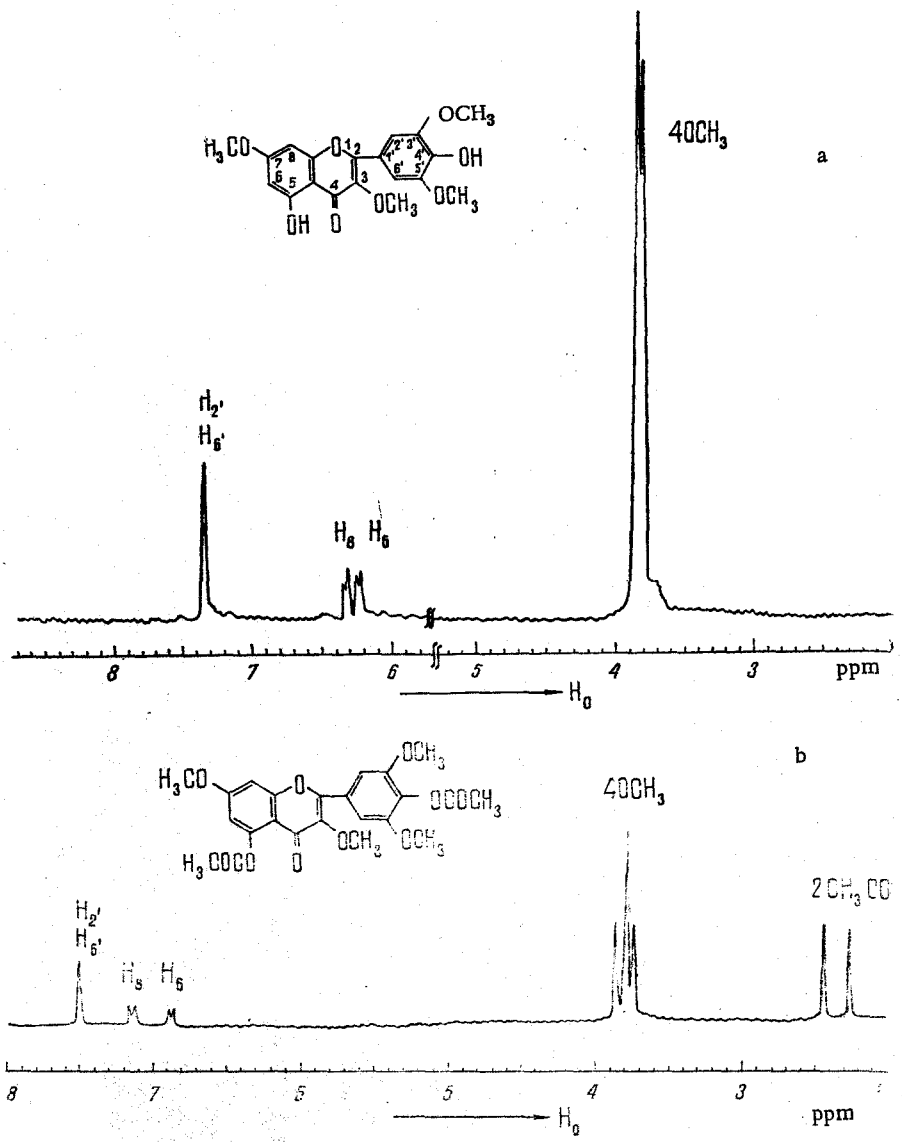


Fig. 1. NMR spectrum of the TMS ether of substance F-II (a) and of its acetate (b).

flavone. We have isolated all these substances previously from the extracts obtained with liquefied CO_2 [1].

Uvaol. Petroleum ether–diethyl ether (1:1) eluted white verticillate crystals with mp 220-221°C (from ethanol) with the composition $C_{30}H_{50}O_2$, $[\alpha]_D^{20}$ +63.7° (c 3.0, chloroform)

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

NMR spectrum, δ, ppm: signal at 5.2 (CH=C), two d's with superposition of a multiplet at 3.2-3.8 with an intensity of 3 H (CH_2OH and CH–OH), s and d in the 0.8-1.1 region (CH_3–C–; CH_3–CH–).

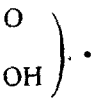
Uvaol Acetate. The reaction of uvaol with acetic anhydride in pyridine at room temperature followed by extraction from the reaction mixture with chloroform yielded a vitreous product with mp 70°C having the composition $C_{34}H_{54}O_4$.

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

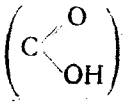
NMR spectrum, δ, ppm: m 5.2 (CH=C), two d's at 3.5 and 4.0 (CH_2OCO), m 4.45 (CHOCO).

Ursolic Acid. After its treatment with petroleum ether, the raw material was steeped in diethyl ether at room temperature three times for a day each time, and the residue after the solvent had been driven off was chromatographed on silica gel 100/250 μ and elution was

performed with petroleum ether, petroleum ether–diethyl ether, and diethyl ether. The diethyl ether eluates gave white crystals with mp 232-240°C, which were then crystallized again, after which they melted at 265-275°C. The crystals obtained were washed with petroleum ether and chloroform and were recrystallized from ethanol: mp 280-283°C, composition $C_{30}H_{48}O_3$.

IR spectrum, ν_{max}, cm^{-1}: 3400-3460 (OH), 1690 and w 2740, 2670, 2680 ().

NMR spectrum, δ, ppm: signal at 5.45 (CH=C), t 3.35 (CH–OH), s and d in the 0.8-1.2 region ($5CH_3$–C; $2CH_2$–CH).

Ursolic Acid Acetate. A solution of 0.3 g of ursolic acid in 2 ml of pyridine was treated with 1 ml of acetic anhydride and the mixture was left at room temperature for a day. Then it was diluted with water and extracted with chloroform, and the residue after the elimination of chloroform (mp 160-180°C) was chromatographed on neutral alumina (activity grade IV), petroleum ether–diethyl ether (8:2) eluted colorless crystals with mp 280-285°C having the composition $C_{32}H_{50}O_4$. IR spectrum, ν_{max}, cm^{-1}: 1740 (OCO), 1690 and w 2740, 2670 (). NMR spectrum, δ, ppm: m at 5.2 (CH=C), t 4.5 (CH–OCO), s 1.95 (CH_3–CO–), s and d at 0.7-1.2 ppm ($5CH_3$–C; $2CH_3$–CH).

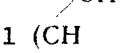
Methyl ursolate was obtained by the action of diazomethane in ether. The reaction product was chromatographed on neutral alumina (activity grade IV). Petroleum ether–diethyl ether (8:2) eluted white crystals with mp 166-169°C having the composition $C_{31}H_{50}O_3$.

The acetate of methyl ursolate was obtained by the action of acetic anhydride in pyridine. Colorless crystals with mp 242-245°C (from ethanol), composition $C_{33}H_{52}O_4$.

Extraction with 50% Ethanol. The comminuted raw material (1 kg) was steeped in 50% ethanol at room temperature three times for a day each time, and after this the extract was evaporated to half its volume and was filtered, and the filtrate was treated three times with petroleum ether and 15 times with diethyl ether. The residue after the elimination of the diethyl ether was chromatographed on polyamide and was eluted with chloroform and with chloroform–methanol.

Taraxerol. Fractions 1-3 of the chloroform eluates were rechromatographed on silica gel L 100/250 μ. Petroleum ether–diethyl ether (9:1) eluted a waxy mass which was washed with chloroform. The chloroform filtrates deposited white crystals with mp 282-284°C having the composition $C_{30}H_{50}O$.

IR spectrum, ν_{max}, cm^{-1}: 3450 (OH).

NMR spectrum, δ, ppm: br. sig. at 5.5 (CH=C), t at 3.1 (), s and d in the 0.7-1.1 region (CH_3–C), (CH_3–CH).

3,3'7-Tri-O-methylquercetin. Petroleum ether–diethyl ether (1:1) eluted yellow crystals with mp 147-149°C (from ethanol) having the composition $C_{18}H_{16}O_7$. The diacetate of the substance was obtained by reaction with acetic anhydride in pyridine at room temperature, after dilution with water a precipitate deposited which was washed with water and was recrystallized from ethanol: mp 175-177°C, composition $C_{22}H_{20}O_9$.

4',5'Dihydroxy-3,3',5',7-tetramethoxyflavone. The ethereal eluates yielded yellow crystals with mp 188-190°C (from ethanol) with the composition $C_{19}H_{18}O_8$.

The diacetate was obtained by the method described above; mp 221-222°C (from ethanol), composition $C_{23}H_{22}O_{10}$.

Fraxetin. Fractions 4-5 of the chloroform eluates from the polyamide deposited yellow crystals on standing, and after recrystallization from alcohol the substance had mp 228-230°C, composition $C_{10}H_8O_5$.

IR spectrum, ν_{max}, cm^{-1}: 3450-3330 (OH), 1715 (C=O), 1600, 1580, 1570, 1510 (C=C).

UV spectrum, λ_{max}, nm: 210 and 350, log ε 3.6 and 4.0, respectively.

NMR spectrum, δ, ppm: s at 3.95 with an intensity of 3 H (CH_3—O—C—), two d's at 6.2 and 7.6, H_3 and H_4, s at 6.5, H_8.

Quercetin and Hyperoside. In column chromatography of the ethereal extract on polyamide, chloroform—methanol (9:1) eluted a crystalline mixture of two substances, which was rechromatographed on silica gel L 40/100 μ. Petroleum ether—diethyl ether (1:9) eluted yellow crystals with mp 312-315°C (from ethanol) having the composition $C_{15}H_{10}O_7$, the NMR spectrum of the substance was identical with that of quercetin.

Diethyl ether—ethyl acetate—methanol (6:2:2) eluted dark crystals which, after two recrystallizations from ethanol, had mp 224-227°C.

The NMR spectrum was identical with that of hyperoside.

Extraction with Methanol. The above-described raw material (1 kg) was steeped with methanol at room temperature three times. The extract was evaporated to 1/3 of its initial volume and the precipitate that it deposited (about 100 g) was filtered off and was then dissolved in chloroform and chromatographed on silica gel L 100/250 μ. Elution was carried out with petroleum ether, diethyl ether, and mixtures of them. The 8:2 mixture eluted β-sitosterol in the form of white crystals with mp 138-139°C, composition $C_{29}H_{15}O$.

IR spectrum, ν_{max}, cm^{-1}: 3430-3320 (OH), 1645, 1465, and 1380 (C=C).

NMR spectrum, δ, ppm: s and d at 0.7-1.2 (CH_3—C, CH_3—CH), t at 3.65 (CH—OH), and 5.3 (CH=C).

Uvaol and ursolic acid were obtained from the subsequent fractions.

SUMMARY

From the herb *Ledum palustre* L. we have isolated β-sitosterol, the triterpenoids taxerol, uvaol, and ursolic acid, the coumarin fraxetin, the known flavonoids quercetin, hyperoside, and 3,3',7-tri-O-methylquercetin, and a new natural flavonoid for which the structure of 4',5-dihydroxy-3,3',5',7-tetramethoxyflavone is suggested.

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