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A GAS-CHROMATOGRAPHIC METHOD OF DETERMINING LEDOL IN THE ESSENTIAL OIL, LEAVES, AND HERBAGE OF *Ledum palustre*

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UDC 543.8.51:665.526.15.002.3

The herb *Ledum palustre* L. (crystal tea ledum; march tea) has long been used in medicine. It is considered that the therapeutic action of this plant is connected with the terpenoid compounds present in the essential oil [1]. The amount of the essential oil and its composition depend strongly on the vegetation phase, the site and conditions of growth, etc. In view of this, the dependence of the amount and the physicochemical properties of the essential oil have been studied as functions of the times of collection, the vegetation phase, the growth site, and the plant organ, and the composition and structure of individual components of the oil have also been investigated [2-7].

It has been found that the predominating amount of essential oil is concentrated in the leaves, and the amount of essential oil in the other organs of the plant is insignificant. The greatest amount of essential oil is found in the leaves in the phase of the ripening of the fruit [2-7]. This is the time (August-September) at which it is recommended to collect the raw material [8].

The main components of the essential oil of *L. palustre*, which has a complex chemical composition, are palustrol, ledol, and (in young leaves) myrcene, and there are smaller amounts of p-cymene, geranyl acetate, hydrocarbons, etc. In the essential obtained from fresh leaves of *L. palustre* collected in Finland, 60 components (60 peaks on a chromatogram) have been detected, 18 of which predominate, the others being present in very small amounts (less than 1%) [9].

The amount of the main components — palustrol and ledol — falls in the period of the shedding of the old and the putting forth of the new leaves, and then it rises again, reaching its maximum in September. There is a hypothesis that one of the pharmacological effects of the herb *L. palustre* is due to the ledol [10], a bicyclic sesquiterpene alcohol with the composition $C_{15}H_{25}O$, mp 105-106.5°C.

We have developed a method for the quantitative determination of ledol in the essential oil of *L. palustre* which is based on the gas-chromatographic separation of the components of the essential oil under programmed temperature conditions in the presence of a known amount of methyl myristate (internal standard). Fairly complete separation (no less than 32 components being detected on the chromatogram) is achieved. The methyl myristate peak does not mask any of the peaks of the essential oil, as has been checked on a large number of samples (more than 100) of the essential oil obtained from the leaves of plants of various vegetation phases and from different growth sites.

On chromatograms of samples of essential oil with a high ledol content the largest peak is that of palustrol, the ledol peak being considerably smaller, and the peaks of the other

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 715-718, November-December, 1978. Original article submitted July 25, 1978.

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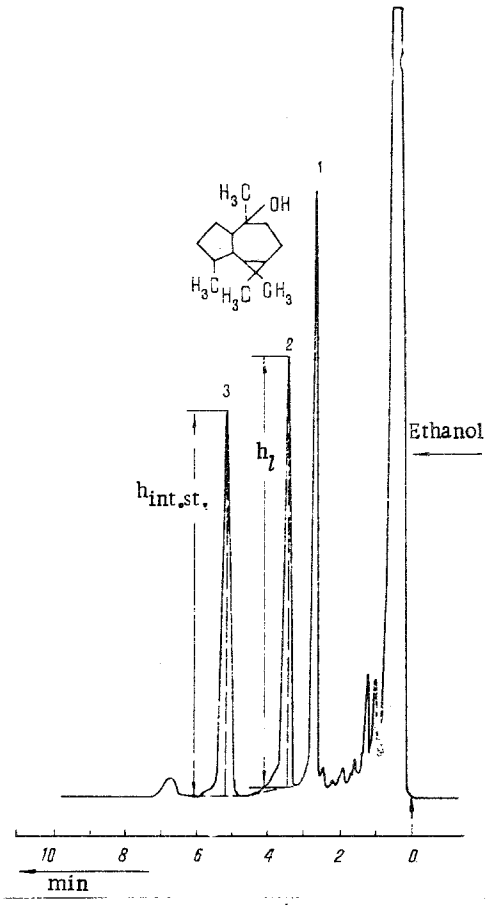

Fig. 1. Chromatogram of the essential oil of *L. palustre*: 1) palustrol; 2) ledol; 3) methyl myristate (internal standard).

components are an order of magnitude smaller still, as a rule (Fig. 1). This simplifies the separation, enables the process of chromatography to be accelerated, and reduces the time of analysis for a series of samples of essential oil with similar ledol contents. The reliability of the method has been checked by the addition of ledol to the essential oil followed by the determination of the total amount of ledol and, moreover, by the preparative isolation of ledol from 20 g of essential oil by freezing out. It was found that the ledol is frozen out incompletely from the essential oil — the residual amount of ledol in the essential oil subjected to the freezing-out process is 9-10%. The results of the preparative isolation and gas-chromatographic determination of the isolated ledol agree well. Since the stems contain practically no essential oil, in order to obtain uniform evaluation of the raw material for its content of essential oil and ledol, we used only the leaves. More than 100 samples of essential oils from the leaves of *L. palustre* from various growth sites in the Central Zone of the European part of the USSR in the early vegetation phases have been analyzed by the gas-chromatographic method. Each sample of oil was obtained from 30 g of air-dried *L. palustre* leaves by steam distillation, using the method of the State Pharmacopoeia of the USSR [8], for 5 h (until the cessation of the distillation of the essential oil). The time of storage of the raw material in the laboratory did not exceed 4 months. As compared with other recommended methods of distillation, this method ensured the most complete distillation of the ledol and other components from raw material with a high ledol content.

The results of the analyses of these samples of essential oils [11] show that the amount of ledol in the essential oil of *L. palustre* varies between 23 and 37% according to the vegetation phase and the growth site. The average content is 25-30%. If the amount of ledol in the essential oil is recalculated to the weight of leaves or herbage taken for obtaining the essential oil it is possible to determine the amount of ledol in the leaves or herbage. The amount of ledol in the leaves during the vegetation period ranges from 0.55 to 1.31% (young leaves) and from 0.13 to 0.62% (leaves of the previous year). The highest ledol content is found in the phase of the ripening of the fruit, and the lowest in the flowering phase [11]. The mean ledol content calculated on the raw material (herbage of *L. palustre*) is 0.25-0.3%.

EXPERIMENTAL

Conditions for Chromatography. Glass column 1.2 m long internal diameter 2 mm filled with 0.6% of poly(ethylene adipate) on Chromosorb W AW 60-80 mesh. Temperature of the column programmed from 100 to 150°C at the rate of 5 deg/min. Temperature of the evaporator 180°C. Rate of flow of gases (ml/min): nitrogen — 60; hydrogen — 40; air — 400. Speed of the paper strip of the recorder 10 mm/min. "Khrom-4" chromatograph, flame-ionization detector.

Description of the Method. About 0.1 g (accurately weighed) of the essential oil of *L. palustre* and about 0.03 g (accurately weighed) of methyl myristate (internal standard) are placed in a 25-ml flask with a tightly fitting glass or polyethylene stopper, 10 ml of ethanol (100-fold amount in relation to the weight of essential oil taken) is added, and the mixture is stirred until the components have dissolved completely. Of the resulting solution, 1-2 μl is transferred to the evaporator of the gas chromatograph with a flame-ionization detector. Not less than three gas-chromatographic analyses of the sample are performed and the heights of the peaks are measured with an accuracy of ±0.5 mm. In the analysis of essential oils with a low ledol content (fluid liquid oils) the weight of methyl myristate is decreased (but not more than twofold) and for oils with a very high ledol content it is increased.

To select an average sample of essential oil with a high ledol content (viscous oil with deposited crystals of ledol), a sample of oil in a tightly closed bottle was heated until it had melted completely and was rapidly cooled. Rapid cooling led to a homogeneous consistency. The sample of oil was taken immediately after this. In order to determine the calculation factor, the gas-chromatographic analysis of a standard mixture of chemically pure ledol and of methyl myristate (internal standard) was performed in parallel under the same conditions as in the analysis of the sample of *L. palustre* essential oil.

In the preparation of the standard sample, about 0.1 g (accurately weighed) of chemically pure ledol and about 0.12 g (accurately weighed) of methyl myristate (internal standard) were placed in a 50-ml flask and dissolved in 40 ml of ethanol. The storage life of the standard solution is not less than six months. This ratio of ledol, internal standard, and solvent ensures approximately equal heights of the ledol and methyl myristate peaks on the chromatogram and also approximately equal concentrations of the internal standard in the reference solution and the sample of essential oil being analyzed, which substantially decreases the error of measuring the heights of the peaks.

The standard solution (1-2 μl) was introduced into the evaporator of the gas chromatograph alternately with samples of the essential oil being analyzed. The heights of the ledol and methyl myristate peaks on the chromatogram must be not less than 100 mm and the separation factor not less than 1, and the adjacent peaks must not interfere with the determination of the heights being measured.

The amount of ledol in a sample of the essential oil of *L. palustre* were calculated from the formula

$$P_l = \frac{P_{\text{int. st}} \cdot h_l}{h_{\text{int. st}} \cdot F},$$

where P_l is the initial weight of ledol, g; $P_{\text{int.st}}$ is the weight of internal standard, g; h is the height of the peak, mm; and F is the calculation factor.

The calculation factor F is found from the standard mixture as the mean of (not less than) three gas-chromatographic replicates by means of the formula

$$F = \frac{P_{\text{int. st}} h_l}{P_l \cdot h_{\text{int. st}}},$$

where the symbols are the same but relate to the parameters of the peaks and the weights of the substances of the standard mixture.

The amount of ledol in the essential oil, x, is found as the mean of three gas-chromatographic replicates from the formula

$$x = \frac{P_l \cdot 100}{P_m},$$

where P_m is the weight of the essential oil.

Statistical treatment of the results of analysis shows that the error of the determination at the 95% confidence level does not exceed ±3.5% with three replicates.

SUMMARY

1. A gas-chromatographic method has been developed for the quantitative determination of ledol in the essential oil of *Ledum palustre* which permits the amount of ledol in the leaves and herbage to be determined.

2. Depending on the vegetation phase and the growth site of the *L. palustre*, the amount of ledol in the essential oil ranges between 23 and 37%. When ledol is obtained from the essential oil of *L. palustre* by freezing-out, the residual concentration of ledol in the essential oil subjected to the process is 9-10%.

3. The mean content of ledol calculated on the raw material (herbage of *L. palustre*) is 0.25-0.3%.

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