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SHED PLANE LEAVES AS A SOURCE OF α-TOCOPHEROL

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A natural source of α-tocopherol — shed plane leaves — has been found. The dynamics of the accumulation of α-tocopherol over the vegetation period of the plant has been studied, and a method has been developed for its isolation and quantitative determination.

α-Tocopherol is the most important natural representative of the tocopherol group. In tissues it fulfills the role of a biological antioxidant and is important for maintaining the structural integrity and functional activity of cell membranes and subcellular organelles.

At the present time, a mixture of α-tocopherols obtained both synthetically and from plant oils is used in medical practice [1]. Natural α-tocopherol is the most active.

We have previously developed a method for isolating α-tocopherol from the leaves of the cotton plant, where it makes up 0.06-0.09% of the air-dry mass (ADM). Continuing our investigations on the isolation of secondary metabolites (SMs) from plant raw material and revealing their roles, we have studied the components of shed plane leaves and have detected a high level of α-tocopherol in them. The use of this raw material as a natural source of α-tocopherol will permit the solution of the problem of supplying it to the medicinal industry and also that of the utilization of plant wastes.

In order to study the dynamics of the accumulation of α-tocopherol in plane leaves, we isolated the total extractive substances from green and shed leaves at various periods: July, August, September, and November (Fig. 1).

A visual comparison of the total extractive substances and available pure samples by TLC and by quantitative mass-spectrometric analysis showed that in the early period (July) the total sterol content (0.34% on the ADM) considerably exceeded the α-tocopherol content (0.27% of the ADM). Later (September, October), the level of α-tocopherol in the total material amounted to 4.4% (of the ADM of shed leaves) and in a still later period (November) the amount of its oxidized forms — α-tocopherylquinone (**1**) and epoxytocopherylquinone (**2**) — had increased (0.17%), with a simultaneous decrease in the amount of pure α-tocopherol (0.27% of the ADM). The oxidized forms were identified by mass spectrometry.

Mass spectrum, *m/z* (%): (**1**) — 444 (M^+, 2), 400 (18), 219 (3), 191 (8), 134 (100); (**2**) — 462 (M^+, 1), 444 (6), 419 (54), 402 (26), 237 (54), 167 (100).

To develop a method for isolating α-tocopherol, we used such solvents as ethanol, chloroform, hexane, and ethanol–chloroform (1:1), the total yields of extractive substances amounting to 10, 7, 5, and 17%, respectively. Although the yield was a maximum on the use of ethanol–chloroform, the total extractive material was contaminated with ballast substances interfering with the subsequent purification of the α-tocopherol. As a result of the investigations performed, we chose ethanol as the extractant.

After the extraction of the air-dry comminuted leaves with ethanol, the alcoholic extract obtained was evaporated to dryness. The residue was treated with chloroform and was separated with the aid of column chromatography. Fractions containing from 5 to 54% of α-tocopherol were isolated. Its yield amounted to 1.5% (on the ADM).

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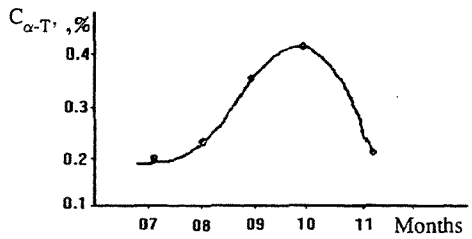

Fig. 1. Dynamics of the accumulation of α-tocopherol.

To raise the yield and improve the purity of the α-tocopherol we have developed a method by which α-tocopherol with a purity of 96-100% has been obtained with a yield of 0.26-0.30% on the ADM [2].

To monitor the level of α-tocopherol in the various fractions of the total extractive material we have developed an appropriate method of quantitative evaluation with the aid of HPLC on a Milikhrom instrument:

Volume injected, μl	3	4	5	7	10	12	15	17	20
Amount of α-tocopherol, μg	0.3	0.4	0.5	0.7	1.0	1.2	1.5	1.7	2.0
Mean value of the area under the chromatographic peak, mm^2	31	71	91	137	290	350	470	545	630

EXPERIMENTAL

α-Tocopherol was isolated by the method of [2]. It was identified by its mass spectrum [3], and also by comparison with a standard by TLC and by the qualitative reaction with the iron-pyridine reagent (a 0.25% solution of α,α-bipyridyl and a 0.1% solution of ferric chloride in ethanol). Its purity was determined on an LP 223 liquid chromatograph (Czechoslovakia) at λ_{max} 294 nm, the solvent being benzene–ethyl acetate (24:1). Column 3.3 × 150 mm; adsorbent Separon FGX-18; pressure 2 atm.

The quantitative determination of α-tocopherol in the fractions was made on a Milikhrom HPLC with detection from the absorption at 294 nm. Column 2 × 60 mm filled with the sorbent Silosorb 600. The sensitivity of the recorder scale was 50.0 mV. The rate of feed of eluent was 200 μl/min, and the chart speed 1800 mm/h. The volume of the sample injected into the chromatograph was 3-20 μl. The retention time of α-tocopherol, using the eluent hexane–isopropanol (10:1), was 55 sec.

A standard solution of α-tocopherol with a concentration of 0.1 μg/μl was prepared by dissolving 10 mg of α-tocopherol in 100 ml of eluent, transferring the solution quantitatively into graduated measuring pycnometers with ground stoppers and making it up to the mark. Each selected solution was prepared in triplicate. We determined the mean value of the area under the chromatographic peak (the results are given above, a linear relationship being shown between the area under a peak and the amount of α-tocopherol in the volume of solution injected into the chromatograph).

With aid of a rotary evaporator, the fractions of the total extractive material from the plane leaves under investigation were evaporated to dryness and were brought to constant weight. Accurately weighed portions of about 10 mg of the oily samples were taken and were transferred quantitatively into 10-ml measuring graduated test-tubes with ground-in stoppers.

Fractions with a high α-tocopherol content were diluted with the eluent to 50-100 ml, and 5- or 10-μl portions were injected into the chromatograph. Solutions of the sample under investigation with a lower content of α-tocopherol (concentrations of about 2 μg/ml) were prepared by taking accurately weighed samples (10 mg) and dissolving them in 5 ml of eluent. Volumes of 5 μl were injected into the chromatograph. After the amount of α-tocopherol in 5 μl had been calculated, the concentration of α-tocopherol in the sample under investigation was determined from the following formula:

$$C_{mg/g} = \frac{L \cdot V}{V_1 N} \cdot 1000,$$

where L is the amount of α-tocopherol in the volume of the sample solution injected into the chromatograph, V_1 is the volume added to the chromatograph column, μl; V is the total volume of sample solution obtained, ml; N is the weight of the sample, mg; and, 1000 is the factor for recalculating ml to μl.

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